HETEROBLASTIC LEAF MORPHOLOGY IN JUVENILE PLANTS OF *DICRANOPTERIS LINEARIS* (GLEICHENIACEAE)

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The peculiar leaves of Gleicheniaceae are a familiar sight throughout the tropics. Their growth varies from low, nearly flat ground covers in exposed savannahs or grassy upland regions to enormous climbing masses of low rain forest areas. In Manus, Admiralty Islands, for example, the writer observed on the steep forested slopes of the Lorengau River leaf masses 150 feet high scrambling over the trees (Wagner, 1947, pl. 5). On a later visit there, Mr D. F. Grether estimated the length of a single leaf to be perhaps "as much as 100 feet in length". Holttum (1954) reports *Dicranopteris linearis* var. *altissima* from Johore as having main rachises up to 1 cm in thickness and "climbing to a height of 30 m". The usual habit lies somewhere between the decumbent and dwarfed growth of exposed places and the tree-climbing growth of rain forests; and the leaves usually form a thicket, often difficult to penetrate.

The seemingly limitless dichotomous growth of these leaves is accompanied by a more or less complete arrestment or abortion of successive axis tips and the outgrowth of lateral pinnae, which in turn repeat the process. Usually a small "bud" may be detected in each fork, but in one form, *Sticherus flagellaris* var. *bracteata*, the axis-tips completely disappear so that the leaf branching conforms to a perfectly dichotomous system (Wagner, 1952, fig. 1).

Although writers have discussed the heteroblastic series leading to leaf dichotomy in Gleicheniaceae, I find no detailed description of any species. Troll (1938) described in general the branching of the leaf and illustrated a few examples of juvenile leaves. Bower's brief treatment of "*Gleichenia* (*Dicranopteris*) *linearis*" (1926, fig. 473), based presumably on the species discussed here, diverges so much from the present observations that it seems desirable to place them on record.

The material includes over 300 tiny plants of *Dicranopteris linearis* removed from freshly exposed banks near Kilauea Crater in Hawaii National Park by Mr Eugene Horner. This wealth of specimens makes it possible to describe the heteroblastic changes in some detail and to gain an idea of the variation. In addition, I will describe the peculiar morphology of the "emarginate" segments.

Examples of the heteroblastic leaf variation in this species are shown in Fig. 2. There is only a broad correlation between size and shape of leaves. Specimens fully three times the size of others may have the same basic form. The changes which take place in progressively more complex leaves of a large series of juvenile plants may be described roughly as follows: From the smallest, pinnately constructed, but merely lobed, leaves, there is a general increase in size accompanied by pinnation in the basal part of the blade to form two pinna pairs. In later leaves, the lowest pinna pair (or sometimes two or three pairs) becomes much exaggerated in relation to the rest of the blade, producing a broadly triangular blade outline. Additional development sees still more enlargement of the basal pinna pair, and abrupt cessation of midrib growth. The midrib becomes

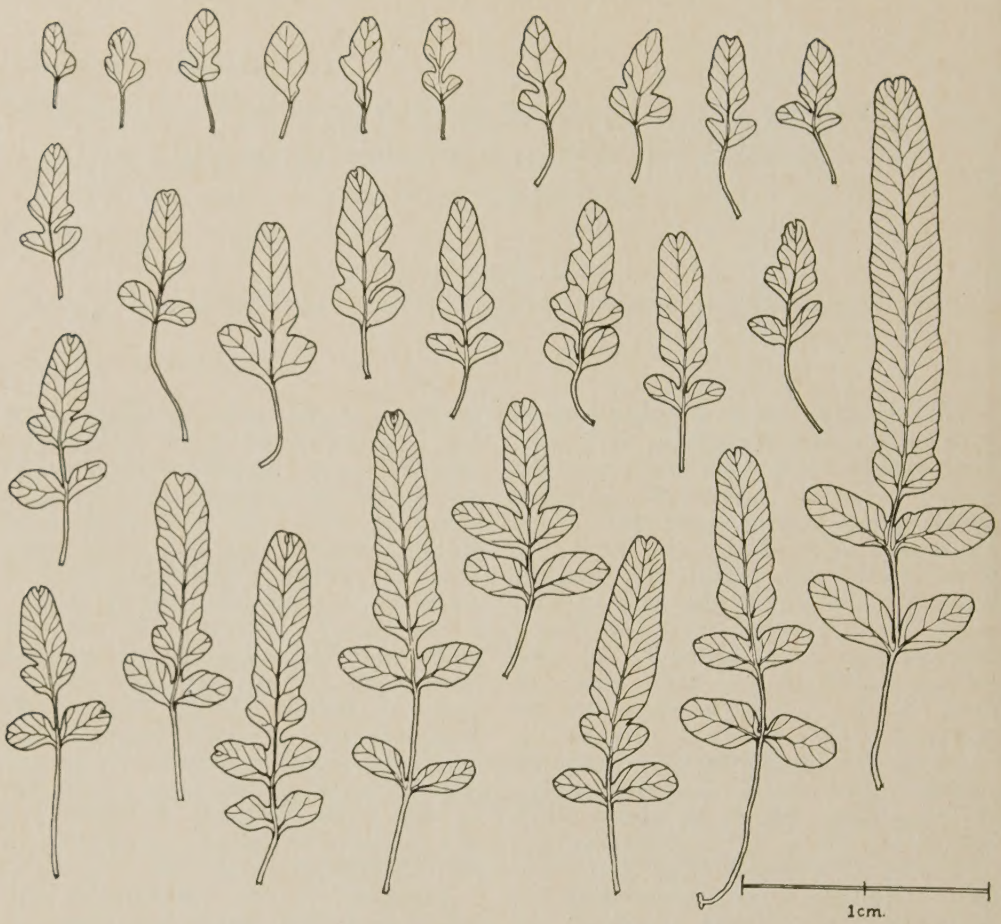

FIG. 1 — Early midribbed stage of *Dicranopteris linearis* from lobed to pinnate conditions.

reduced to a small, scaly papilla. Finally, the mature rhizome forms leaves with the more familiar gleichenioid architecture; the basal pinnae form a succession of dichotomies. The heteroblastic variation is so great, and the successive "stages" so gradual, that the description of "stages" must be considered arbitrary and largely a matter of convenience.

STAGE I: EARLY MIDRIBBED STAGE — The first leaves of the young sporophyte of *Dicranopteris* are pinnately constructed and possess a well-defined midrib, the condition found also in *Botrychium* (Ophioglossaceae) and *Nephrolepis* (Davalliaceae). More commonly, among ferns, the earliest leaves lack midribs and are dichotomous in organization. As illustrated in Fig. 1, the most minute leaves, only 3 mm in length, may be almost without lobulation. But soon, in the succession, one or two pairs of lobes or pinnae appear, these forming the broadest part of the blade. In general, the basal lobes rarely spread more than two or three times the breadth of the main part of the blade. In larger leaves with this overall plan, the number of lateral pairs of lobes or pinnae is two, although the largest, 40-50 mm in length, may have incipient cutting leading to a third pair of lobes or pinnae.

STAGE II: LATE MIDRIBBED STAGE — The late midribbed stage is distinguished

by amplification of the lowest pinna pair, but there is complete transition between " Stage I " and " Stage II " in this respect. Also, small leaves in the size range more typical of " Stage I " only 15 mm in length may have the blade shape of " Stage II ". The enlargement commonly involves not only the lowest pinna pair but the second (and rarely the third) pinna pair as well; in fact, some individuals have second pinna pairs which are more greatly emphasized than the bottom pairs. The outline of the leaves at this stage varies from equally to broadly triangular and there is a general correlation of the broadening in outline with increase in the robustness of the leaf. In the most extreme form, the late midribbed stage is a tripartite leaf with two very large lateral pinnae and a smaller central unit, and the blade outline is more than three or four times as wide as long.

In the larger leaves of the late midribbed sequence, the first semblance of the mature segmentation appears on the pinnae in the tendency toward a regular " pectinate " lobation. The modifications of " Stage II " seem to be a " preparation " for the following stages, in which dichotomous organization sets in. This is evidenced by (a) the gross amplification of the basal pinna pair, and (b) the reduction in relative size and complexity of the central (i.e., midribbed) unit. Some intermediates to the next stage are found in which the central part of the blade grows approximately one-half or two-thirds of its full development but fails to complete its growth.

Stage III: First Midrib Abortion Stage — At this point in the heteroblastic series, the leaf axis simply becomes completely arrested after producing two large lateral pinnae. This abortion of growth may occur in leaves of various sizes, some of them smaller than the largest of the later midribbed forms. The failure of the midrib to unfold results in a small papilla or meristem, invested with shiny, mahogany-colored trichomes. This " bud " probably remains entirely quiescent in most leaves; but it may grow out again after the basal pinnae have matured and form a second pinna pair with an arrested apex at the new level. It is in this midrib-abortion stage that wholly typical pectinate segmentation of the mature leaf first appears. However, the smaller leaves in which midrib arrestment has occurred may have pinnae with merely wavy margins, or even perfectly entire margins like some of those depicted in Fig. 2. Again the correlation between the morphology and size is not close, as mentioned above: the smallest leaves showing failure of midrib growth may have petioles only 20-25 mm long and entire-margined pinnae only 12-15 mm long. But typical cessation of midrib activity was not found in leaves any smaller than this, although Bower's illustration (l.c.) shows it in leaves of approximately the morphology of early " Stage I ". Bower may have been dealing with another member of the Gleicheniaceae, or his specimens may have been damaged by exposure and had failed, therefore, to complete their normal growth (*cf.* his figs. c, d, and e).

The lateral pinnae during the first midrib abortion stage are determinate structures which complete their growth to a long tip without lateral outgrowths.

Stage IV: Mature Stage — At this stage, axis abortion effects not only the central midrib but the axes of the lateral pinnae and their derivatives as well. This is the familiar growth pattern of the leaves of *D. linearis* and it will not be figured or described here in any detail. Successive lateral pinnae form large basal auricles, and elaminate gaps between dichotomies produce a series of naked axes until ultimately the wholly laminate " pinnae " are reached (as illustrated by Troll, 1938, fig. 1232; Bower, op. cit., fig. 474; and Holttum, 1954, fig. 141). The arrested main axes may resume activity (as shown by Holttum, op. cit., fig. 16c). The major differences of the mature leaf morphology from that of the juvenile leaf in the earliest midrib abortion stage are (a) the indeterminate rather than determinate basal pinnae; (b) the formation of naked, elaminate axis sectors; and (c) the seemingly unlimited growth potential.

Apical Structure of Segments — Apparently all specimens of *Dicranopteris linearis* and its forms (including the hairy

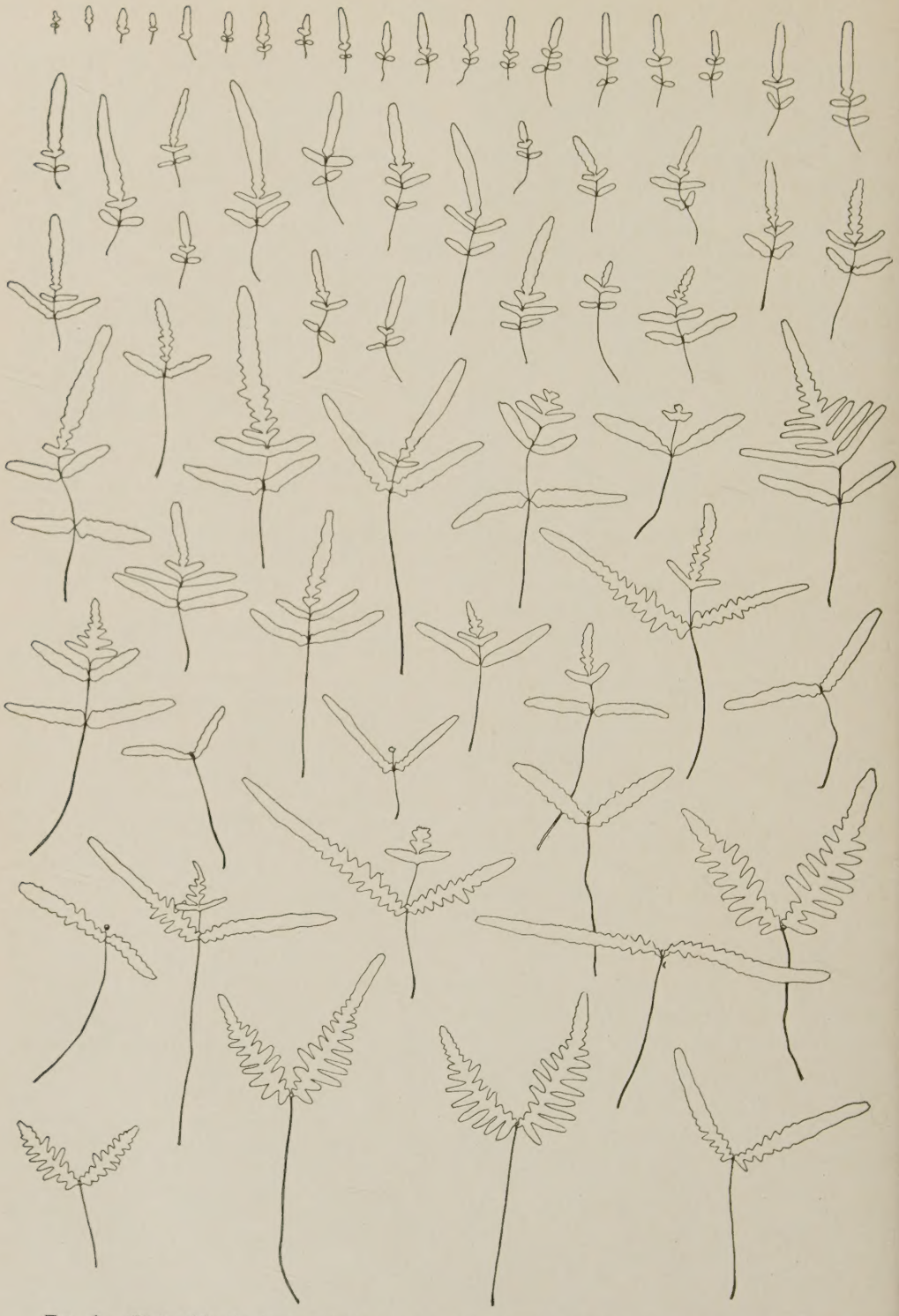

FIG. 2 — Heteroblastic leaf variation in juvenile plants of *Dicranopteris linearis* illustrating changes from midribbed to midrib arrestment stages. × 0·7.

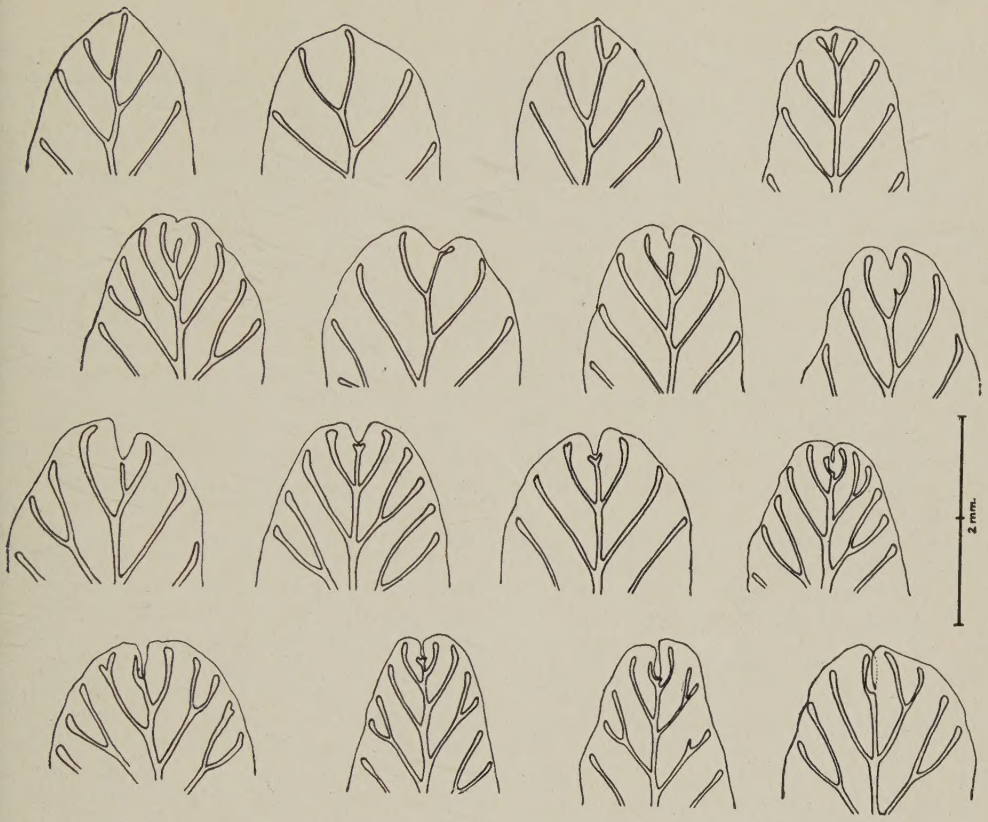

FIG. 3 — Tracings of cleared segment tips showing the variation of venation and margins from normal to notched.

Hawaiian type treated by authors as a variety or species) show a peculiar notching of the segment tips. The notching is variable. I have seen some specimens in which practically all the divisions are notched and others in which only a few are notched. In mature leaves the sinuses may be up to 1·5 mm deep.

This "emarginate" condition is evidently widespread through the Gleicheniaceae. It is shown in University of Michigan specimens of *Dicranopteris flexuosa, D. pectinata, Hicriopteris chinensis, Sticherus furcatus,* and *S. bifidus.* In some species, for example the last, the condition is only weakly and sporadically developed.

The sinuses are decidedly "abnormal-looking" for several reasons: (a) many segments, side-by-side with notched ones, have normal apices (such as those illustrated in the top row in Fig. 3); (b) when the notching is expressed, the sinuses have various forms; and (c) the bases of the sinuses appear to be injured or arrested at some early stage of development. The condition of notching appears in the earliest leaves of the heteroblastic series, and the description to follow deals only with juvenile leaves.

The "normal" or non-emarginate segment tips are obtuse but with a more or less distinct point at the apex. Their venation pattern is symmetrical and the ultimate veins at the apex are similar in their development to those which lie proximally. The smallest notches are only 0·05 mm deep but the vein or veins of the tip are imperfectly developed (Fig. 3, second row from top). Deeper

sinuses take various forms, but the V-shaped notch is the most common. Extreme types include the parallel-sided condition and that in which the two sides of the sinuses overlap each other.

Anatomically the normal tip of a lobe possesses a two-layered marginal strip composed of narrow cells 2-8 times as long as broad. This strip runs with little modification around the segment; there is only slight shortening of the cells at the apex proper. Where the tip is notched, however, the organization is changed: The normal marginal cells now extend down the sides of the sinus to its base. At the base there is generally a flat cell mass or small papilla of 5-15 cells. The vein may terminate immediately below the bottom of the sinus, but it may also run up along one or, if branched, both sides of the notch, directly next to the 2-layered marginal strip.

It is tempting to think of the condition as a result of injury, due perhaps to destruction of the apical cell by exposure during the last stages of unrolling of the primordial segment tip. Whatever the cause, it seems very likely that the apical cell either dies or changes its normal activity. It is possible that the emarginate condition may be morphogenetically related to the same process that produces arrestment of the midrib in juvenile leaves and later the lateral axes of mature leaves. The apical cell may simply modify its activity or stop growing when maturation of the segment is nearly complete.

Summary

Heteroblastic origin of the dichotomous growth habit in juvenile leaves of *Dicranopteris linearis* has not previously been described or figured in detail. A collection of over 300 juvenile plants forms the basis of a description of the heteroblastic variation and illustrates the occurrence of peculiar notched segment tips.

There is only a broad correlation between the overall pattern of the juvenile leaf and its size. A specimen having the same morphology as another may be as much as three times as large.

The changes are more or less gradual when observed in a large series of leaves, but four stages may arbitrarily be distinguished: (1) Early midribbed stage — blades lobed or pinnate, the basal pinnate, part of the blade not more than two or three times as broad as the main apical part of the blade. (2) Late midribbed stage — blades pinnate, the one to three basal pinna pairs much exaggerated to produce a more or less triangular blade outline. (3) First midrib abortion stage — blades dichotomous due to cessation of apical growth between the two basal pinnae; basal pinnae determinate and ending in an unmodified tip. (4) Mature stage — blades indeterminate, producing repeated forkings from the lateral pinnae.

Distinctive notching of the segment tips produces small apical sinuses of various forms. The apical cell seems to be arrested (injured?) when the development of the segment tip is almost complete.

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THE UNISEXUAL FLOWER AGAIN — A CRITICISM

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In Heslop-Harrison's recent review of Sex Expression in Flowering Plants (1957), before proceeding to the main topic of his paper, he has some remarks to make on the sexual character of flowers as a whole. This section has the heading —" The origins of monocliny and dicliny ". Personally the terms bisexuality and unisexuality would be preferred, though perhaps not so euphonious. However, this is a minor matter and he explains fully the meaning he attaches respectively to monocliny and dicliny. These terms may be considered a little out of fashion these days. Further, speaking of flowers generally is it not time we ceased using the somewhat cumbersome term hermaphrodite, time-hónoured though it be, and adopted generally in its place bisexual?

Assuming the angiosperms are a monophyletic group he proceeds to write —" it is possible to conceive of the primitive flower as having been either bisexual or unisexual". He then states using his own words — " The majority of floral morphologists who have considered the matter have been inclined to suppose that the bisexual condition was primitive (*cf.* Parkin, 1952), but this view has been challenged in recent years from a number of quarters." In support of this he refers to Sporne's papers. His 1949 paper has already been criticized (Parkin, 1952) showing that his statistical approach does not convince one of the primitiveness of the unisexual condition. Heslop-Harrison ends this paragraph with the sentence —" Similar views are apparently held by Hagerup (1938)."

The paper to which he refers I happen to have by me as I pen this criticism. It has to do with the gynoecium of *Salix cinerea*, in which he shows that the placentae are not borne on the carpels but directly on the receptacle. The ovule is, therefore, an independent organ and comparable morphologically with the stamen. The funicle is held to correspond to the filament of the stamen. The carpels form merely an involucre of barren leaves and do not bear ovules. He has to a great extent McLean Thompson on his side who in his writings has shown himself to be *acarpous* as regards his views on the female part of the flower. Otherwise I am unaware of any general acceptance of such bizarre and revolutionary views respecting the morphology of the flower. To be a little flippant it might be suggested that such investigators are putting as it were phylogeny in reverse. Advanced and at the same time often reduced flowers are studied and what appear to be ovules terminally situated or attached directly to the floral axis are taken as primitively so situated. Working backwards one presumes the ovules move gradually to the margins of the carpels until ultimately we come to the follicle. Surely a *reductio ad absurdum*. But a critic might reply — Oh, angiosperms with terminal ovules and ones borne directly on the floral axis might have followed a different line of evolution from ones with ovules borne on the carpellary margins. This would point to a polyphyletic origin for the group as a whole. He might in this way escape a dilemma but not convincingly!

In the next paragraph Heslop-Harrison writes that the evidence in favour of the polyphyletic origin of the angiosperms is growing and refers to data from anatomy, mentioning papers by Bailey (1951), Cheadle (1953) and Metcalfe (1954). The two former papers have been studied and Dr Metcalfe has kindly let me have on loan the number of the Kew Bulletin

(1954) containing a more complete account of his views than he gave before the recent Paris International Botanical Congress referred to by Heslop-Harrison. The gist of these papers is to the effect that various elements of the xylem, vessels in particular, have most likely arisen several times independently by parallel evolution within the angiospermae. With this I am entirely in agreement. Research here supports the view that the tracheid is ancestral to the vessel element and that the scalariform perforation is a kind of transitional stage. Further, the evidence also points to the earliest angiosperms being vesselless. Cheadle's researches are of particular interest here as showing that vessels must have originated *de novo* in monocotyledons. This brings us to the intriguing question as to the origin of this group. At the present time the view generally held is that the monocotyledons branched off from the dicotyledons at a very early period. Cheadle considers that the vascular evidence points to the monocotyledons as having arisen from woody dicotyledons that lacked vessels. This is contrary to the old view of an aquatic origin and fits in with the general idea now held that in the main trees preceded herbs in dicotyledonous descent. But I am digressing. The time seems ripe, however, for a general review of the phylogeny of the monocotyledons dwelling particularly on the various theories that have been put forward. It would make an interesting and instructive one, and might open out new lines of inquiry. But the point I wish to emphasize in this paragraph of criticism is to the effect that the polyphyletic origin of certain vascular elements within the angiospermae themselves does not invalidate the holding of a monophyletic origin of the group as a whole. In fact they have little bearing on it.

In the next paragraph, a short one, Heslop-Harrison asserts that without doubt two types of dicliny are distinguishable among modern flowering plants. It is here especially that one is inclined strongly to join issue with him.

His first type has been attained by the suppression of pistillate (term carpellate preferred here) structures in the male, and staminate structures in the female. He remarks also that the rudiments of the missing sex are often conspicuous in the flower. With this paragraph I am in general agreement.

As regards his second type he writes "unisexuality seems to be a basic attribute of the flowers". Then he enumerates certain Orders by name and adds "in which regularly hermaphrodite species are either quite unknown or rare". Further, "*the flowers show no vestiges of the missing sex*". There seems here to be some rather loose wording which I have taken the liberty to italicize. The occurrence, however rare, of a hermaphrodite species surely rules out the Order as having been unisexual from the beginning unless one imagines a kind of freak mutation. Then the assertion that the flowers show no vestiges of the missing sex is too sweeping.

Let me now take *in seriatim* the Orders he lists as examples of his second type:

Pandanales — In *Freycinetia* staminodes are present in the female flowers. Both Engler & Prantl and Hutchinson mention and depict them.

Salicales — The supposed primitive unisexuality of this Order was criticized in my former paper (Parkin, 1952). One might add that Lawrence in his recent text-book (1951) sums up for much reduction and at the same time for advancement in these flowers, suggestive of primitive bisexuality in this Order.

Garryales — A rudimentary gynoecium is present in the male flower of the single genus *Garrya*, composing the Order (*see* Moseley & Beeks, 1955, p. 335).

Myricales — *Myrica gale* can be bisexual — in fact the disposition of the sexes in the flowers is very variable.

Juglandales — A rudimentary gynoecium in male flowers may be present in some of the genera.

Fagales — Hutchinson (1926) calls attention to a rudimentary ovary in the male flower of the Fagaceae.

Urticales — In the Ulmaceae the flowers are either bisexual or unisexual. The genus *Ulmus* is generally regarded as the least evolved florally of the Order and it has bisexual flowers. The Moraceae, a more advanced family, is unisexual and the Urticaceae, unisexual and herbaceous,

is regarded in some ways as the most evolved.

We thus see that in the second type of Heslop-Harrison's unisexual flowers there is considerable evidence of the missing sex and it is surely difficult to account for these vestiges other than as pointing to a former bisexual condition of the flower. There seems then no justification for the division of plants with unisexual flowers into two categories — one primitive and the other derivative. The evidence in my opinion points strongly to no existing unisexual flowering angiosperm as being primitively so constructed. All such are regarded as having had bisexual ancestors. Assuming then that primitive angiosperms had bisexual flowers unisexuality has continually been asserting itself in various lines of descent and, one might add, is still doing so. The earlier it has arisen, the more marked will be the difference between the two kinds of flowers and the manner in which they are separately borne on the plant.

Perhaps a slight apology is due for butting in again on this theme, but my excuse is that a student on reading Heslop-Harrison's remarks might conclude that the monophyletic origin of the angiospermae had by recent investigations been seriously challenged and that a strong case had been made out for regarding certain unisexual flowers as primitive. This short paper is to show that the evidence really points the other way and that no unisexual flower extant can be regarded as otherwise than having descended from a previous bisexual one.

Summary

Heslop-Harrison's division of unisexual flowers into two types — one derivative and the other primitive — is criticized and not accepted.

All unisexual flowers extant are held to be derivative and to have had bisexual ancestors.

The fact that vessels and other anatomical features have arisen several times independently *within* the Angiospermae is no argument against the monophyletic origin of this great group as a whole.

The relinquishment of the term hermaphrodite and the use generally of bisexual in its place are recommended.

My grateful thanks are due to Miss Lorna I. Scott, M.Sc., Department of Botany, University of Leeds, for typing the manuscript and for several suggested improvements in the composition which have been gladly adopted.

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A CONTRIBUTION TO THE FLORAL MORPHOLOGY AND EMBRYOLOGY OF *AEGLE MARMELOS* CORREA

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Not much work has so far been done on the embryology of the family Rutaceae (*see* Schnarf, 1931). Souèges (1926) described the development of the embryo in *Ruta graveolens*. Studies on *Citrus* include the investigations of Strasburger (1878), Osawa (1912), Torres (1936), Bacchi (1943), Gurbel (1952) and Banerji (1954). The most extensive work is that of Mauritzon (1935) who studied 25 genera and 30 species.

A special feature is the occurrence of nucellar embryony which has been reported in *Citrus* (Strasburger, 1878), *Xanthoxylum* (Mauritzon, 1935), *Murraya koenigii* (Chakravarthy, 1935), *M. exotica* and *Aegle marmelos* (Chakravarthy, 1936). In the first three cases the adventive embryos progress as far as the differentiation of the cotyledons, while in the other two they become arrested and only the zygotic embryo reaches maturity.

Bacchi (1943) pointed out that in *Citrus paradisi* and *C. aurantium* polyembryony may sometimes be due to the presence of two gametophytes in the same ovule.

The present investigation deals with the life history of *Aegle marmelos*.

Material and Methods

The material, collected locally during 1951-1954, was fixed in formalin-acetic-alcohol and also in Craf. Due to the presence of tannin and mucilage, young buds, flowers, post-fertilized ovaries and seeds were trimmed on the sides to facilitate infiltration.

The customary methods of dehydration and imbedding were followed. Sections were cut at a thickness of 6-10 μ for younger and 12-16 μ for older stages. Iron-haematoxylin as well as safranin and fast green combinations were used for staining and both gave good results. Acetocarmine smears of pollen mother cells, and whole mounts of the endosperm and embryo were also examined.

Several preparations of *Aegle marmelos* were kindly given to us by Dr I. Banerji, Department of Botany, Calcutta University. Figures 32, 92 and 93 have been sketched from these.

Observations

External Morphology — The genus *Aegle* includes five species: *A. sepiaria*, *A. barteri*, *A. decandra*, *A. glutinosa*, and *A. marmelos*. The first four are distributed throughout the world while *A. marmelos* occurs in India, Burma and Ceylon and is an important medicinal plant. It is a deciduous, moderate-sized tree, with straight, short thorns and trifoliate aromatic leaves. The greenish-white fragrant flowers appear from May to June, and the fruits ripen by December. The globose fruit is grey or yellowish with a smooth, hard, aromatic rind. Numerous oblong seeds are embedded in the sweet, orange-coloured mucilaginous pulp. The flowers are borne in axillary panicles. There are 4-5 deciduous sepals and the same number of oblong, thick, and gland-dotted petals. Around the inconspicuous disc are 45-50 stamens whose anthers are hairy in young stages. The multicarpellary, syncarpous gynoecium has 8-20 loculi, each containing numerous ovules in two rows on an axile placenta. The style is short, terminal and deciduous. The stigma is capitate or fusiform.

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Lysigenous oil ducts are present in almost all parts of the plant. Similar ducts are also known in *Citrus medica* (Fohn, 1935).

MICROSPORANGIUM — The anther consists of four sporangia (Fig. 1). Oil glands are irregularly scattered in the lobes (Figs. 1-3). In two cases the anthers showed only two and three microsporangia respectively (Figs. 2, 3).

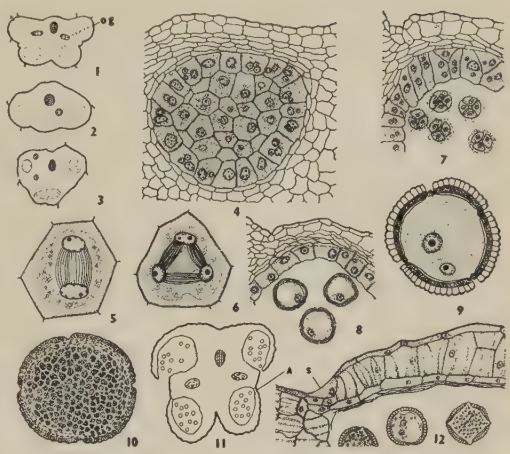
In our material the wall of the anther consists of the epidermis, endothecium, three to four middle layers, and the tapetum (Fig. 4). The epidermal cells are thin-walled and later become greatly stretched and flattened (Figs. 8, 12). At the mother cell stage some of the epidermal cells grow into unicellular hairs but they fall off after the microspores have separated from the tetrads.

By the time the pollen grains become bi-celled, the endothecial cells elongate radially and develop fibrous thickenings (Fig. 12). Occasionally, some of them may divide periclinally. While the outermost middle layer persists, the inner ones collapse at an early stage (Fig. 12).

The richly cytoplasmic tapetal cells undergo mitotic divisions and become 2 to 4-nucleate (Figs. 4, 7). Subsequently, the nuclei fuse giving rise to polyploid masses (Figs. 4, 7). The tapetum is most active during meiosis and persists up to the formation of uninucleate pollen grains (Fig. 8). Gradually its walls break down and the contents are absorbed (Fig. 12).

Banerji (1954) reports that in *Citrus grandis* there are two middle layers; and the tapetum becomes 2 to 3-layered at places. The flattened middle layers are said to persist in the mature anther.

MICROSPOROGENESIS — Before the onset of meiosis, the microspore mother cells enlarge and show a dense granular cytoplasm. Their protoplasts shrink and a special mucilaginous wall is secreted between the protoplast and the original wall (Fig. 4). No wall is laid down after Meiosis I (Fig. 5) but in the equatorial region there is a dense band of cytoplasm which soon becomes inconspicuous. Secondary spindles appear after Meiosis II (Fig. 6). During cytokinesis vacuoles are formed between the daughter nuclei and



FIGS. 1-12 — (*og*, oil gland; *s*, stomium). Fig. 1. Diagram of t.s. of young anther. × 20. Figs. 2, 3. T.s. abnormal anthers with two and three microsporangia respectively (semi-diagrammatic). × 20. Fig. 4. Part of anther lobe enlarged from Fig. 1 to show microspore mother cells. × 200. Figs. 5, 6. Meiosis I and II. × 680. Fig. 7. Part of anther lobe at the time of tetrad formation. × 200. Fig. 8. Same, at uninucleate pollen grain stage. × 200. Fig. 9. Two-celled pollen grain. × 680. Fig. 10. Mature pollen grain in surface view. × 680. Fig. 11. T.s. dehisced anther (semi-diagrammatic). × 20. Fig. 12. Enlarged view of portion *A* in Fig. 11; note the small size and absence of fibrous thickenings in endothecial cells in the region of stomium. × 200.

are followed by centripetal furrows. Wedges of the special mucilaginous wall invade the furrows and meet in the centre, bringing about quadripartition. Both tetrahedral and decussate tetrads are formed (Fig. 7). The enlargement of the microspores is accompanied by an absorption of the mucilaginous wall and breaking down of the original wall of the mother cell releasing the microspores. The wall of the microspore gradually differentiates into the exine and intine (Fig. 8).

MALE GAMETOPHYTE — At first the nucleus lies in the centre of the pollen grain but due to the appearance of a vacuole it is pushed towards the wall (Fig. 8). Here it divides resulting in the organization of a large vegetative and a small lenticular, generative cell (Fig. 9). In *Pilocarpus pennatifolius* (Honsell, 1954) also the mature pollen is said to be binucleate.

The pollen grains are 4 to 5 colporate (Fig. 9) and the exine shows warty sculpturing (Fig. 10). Banerji (1954) states that in *Citrus grandis* they are tetracolpate and are shed at the 3-nucleate stage[1].

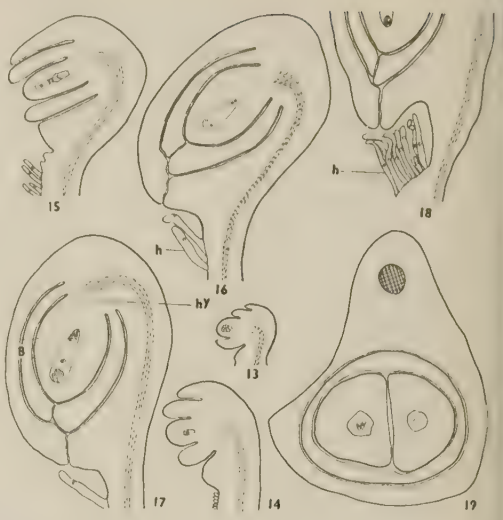
At maturity the two adjacent sporangia do not become confluent and each locule dehisces by a separate stomium (Figs. 11, 12).

The pollen was germinated on sucrose-agar media (1-80 per cent: 1 per cent) with the hanging drop technique. Maximum germination (60 to 70 per cent) was obtained in 40 per cent sucrose. As a rule only a single tube emerges from each grain and shows conspicuous streaming of cytoplasm. The pollen tube reached the maximum length of 160 microns in five hours after sowing. Due to the dense granular contents of the tube the fate of the nuclei could not be traced.

MEGASPORANGIUM — Both the integuments become distinguishable at the time of differentiation of the archesporium. To begin with the outer integument remains arrested on the funicular side (Figs. 13, 14) while the inner one takes the lead and extends beyond the nucellus (Fig. 15). By the time the reduction divisions are over and the functional megaspore has become vacuolated, the integuments are well advanced and form a narrow micropyle with an exostome and an endostome. During the development of the embryo sac, the ovule curves further and becomes anatropous (Figs. 16, 17). At this time the outer integument is 4-5 layered while the inner is 3-4 layered (Fig. 82). One ovule showed twin nucelli with common integuments (Fig. 19).

Honsell (1954) reports that in *Pilocarpus pennatifolius* both the integuments form the micropyle but in *Calodendron capense* only the outer integument is concerned.

The nucellus is massive and there is a prominent parietal tissue. The primary parietal cell as well as the apical cells of the nucellar epidermis undergo repeated



FIGS. 13-19 — (*h*, hairs; *hy*, hypostase). Figs. 13-17. L.s. ovules showing progressive curvature and development of integuments; in Fig. 15 there are two sporogenous cells in a row with a vegetative cell in between them (semi-diagrammatic). × 90. Fig. 18. Upper part of ovule at mature embryo sac stage; funicular hairs come in close proximity of the micropyle (semi-diagrammatic). × 90. Fig. 19. T.s. ovule with twin nucelli and common integuments (semi-diagrammatic). × 90.

divisions (Figs. 22-25). In the Rutaceae, well developed parietal tissue has also been reported in *Melicope ternata, Choisya ternata, Boenninghausenia albiflora, Dictamnus albus, Coleonema album* and *Ruta graveolens* (Mauritzon, 1935), *Citrus grandis* (Banerji, 1954), *Calodendron capense* and *Pilocarpus pennatifolius* (Honsell, 1954).

Soon after the embryo sac has organized, some of the cells at the chalazal end stain deeply (Fig. 17, *hy*). After fertilization they become slightly thick-walled and constitute the 'hypostase' (Figs. 40, 51, 53). The funicular vascular supply terminates below it (Figs. 55, 61).

Some of the placental and funicular epidermal cells elongate into richly cytoplasmic unicellular hairs. They reach as far as the micropyle (Fig. 18) and probably provide nourishment to the pollen tubes.

MEGASPOROGENESIS—The archesporium comprises 2-5 cells (Fig. 20) but as a rule

1. The pollen grains of *Aegle marmelos* are packed with granular contents, and we were unable to ascertain if the generative cell divides before dehiscence.

FIGS. 20-33 — (*p*, polar nuclei). Fig. 20. L.s. young nucellus with multicelled archesporium. × 200. Fig. 21. Same, showing two sporogenous cells. × 200. Figs. 22, 23. Deep-seated megaspore mother cell. × 200. Fig.24. Linear tetrad of megaspores, the upper two megaspores have degenerated. × 200. Fig. 25. Same, chalazal megaspore is functional. × 200. Figs. 26-29. Two-, four- and eight-nucleate gametophytes. × 400. Fig. 30. Organized embryo sac. × 400. Figs. 31-33. Stages showing gradual degeneration of embryo sacs. × 400.

only one of them functions. In Fig. 21 two sporogenous cells are lying one below the other. Mauritzon (1935) also reported a multi-celled archesporium in *Coleonema album, Pilocarpus pennatifolius, Erythrochilton brasiliensis, Boenninghausenia albiflora, Skimmia japonica* and *Triphasia aurantiola*.

The megaspore mother cell (Figs. 22, 23) undergoes the usual reduction divisions and forms a tetrad of megaspores which may be linear (Figs. 24, 25) or T-shaped. The chalazal megaspore functions. Degeneration of the megaspores starts from the apex downwards (Figs. 24, 25).

Within a single ovary the ovules may show all stages from the megaspore tetrad to the 8-nucleate embryo sac. This also happens in *Citrus aurantium* and *C. paradisi* (Bacchi, 1943).

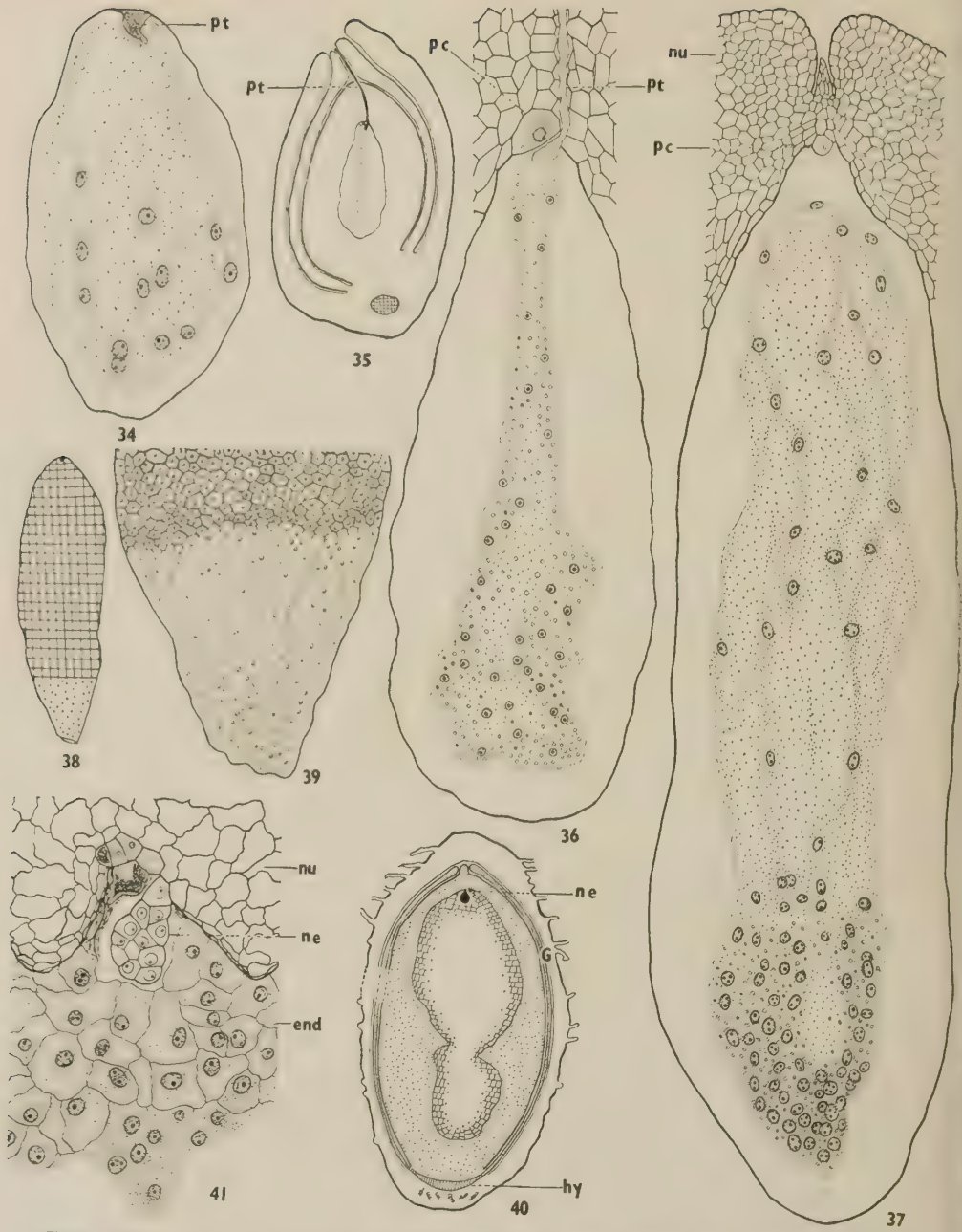
FEMALE GAMETOPHYTE — The functional megaspore undergoes three successive divisions resulting in the formation of 2-, 4- and 8-nucleate gametophytes (Figs. 26-29). The mature embryo sac has the usual organization (Fig. 29). The synergids are hooked and show a prominent filiform apparatus (Figs. 29, 30, 33). The nucleus is often situated at the lower end of the cell (Figs. 29, 30). The polar nuclei come to lie side by side in the upper or middle part of the embryo sac (Figs. 29, 31, 32). The antipodal cells are variable in size and shape (Figs. 29, 30, 33) and are ephemeral. This is also true of *Citrus paradisi, C. aurantium* (Bacchi, 1943), and *C. grandis* (Banerji, 1954).

There are nearly 200 ovules in an ovary of which approximately 75 per cent contain degenerated gametophytes (Fig. 33). Many others have healthy polar nuclei but a degenerating egg apparatus (Fig. 32). Sometimes, the synergids may degenerate while the egg may remain healthy (Fig. 31).

ENDOSPERM — The stigma invariably showed germinating pollen grains and many embryo sacs had remnants of pollen tubes (Figs. 34-36). It may be presumed that double fertilization occurs, but when the egg is in a degenerated state (Fig. 32) it is possible that only triple fusion takes place.

Repeated divisions of the primary endosperm nucleus give rise to a large number of free nuclei (Figs. 34-37, 55, 59, 61, 68) and some of these aggregate at the chalazal end (Figs. 37, 55). Normally the endosperm nuclei are about 12 μ in diameter but in several embryo sacs they measured 18 μ. In one of them the nuclei in the central part were conspicuously larger (25 μ), those in the upper part were smaller (12 μ) and in the lower part of intermediate size (18 μ).

Chopra (1955) observed marked variations in the size of endosperm nuclei in some members of the Cucurbitaceae, and Johri & Garg (1956) in some plants of the Leguminosae. Geitler (1955) reports that abnormally large nuclei occur in the chalazal region of the endosperm in *Allium ursinum*. He draws attention to the fact that these nuclei retain a prophasic structure and become polyploid. They develop continuously without an interphase and become extremely large. The same



FIGS. 34-41 — (*end*, endosperm; *hy*, hypostase; *ne*, nucellar embryo; *nu*, nucellus; *pc*, ' plasma-rich ' nucellar cell; *pt*, pollen tube). Figs. 38, 39. From dissected whole mounts, rest from micro-tome sections. Fig. 34. Free nuclear endosperm. × 333. Fig. 35. L.s. ovule showing the course of pollen tube. × 66. Fig. 36. Same, magnified view of embryo sac with adjoining nucellar cells at the micropylar end. × 333. Fig. 37. Advanced stage of free nuclear endosperm; a ' plasma-rich ' nucellar cell is seen in Figs. 36 and 37. × 150. Fig. 38. Endosperm at the globular stage of embryo; the lower portion is free nuclear while the upper is cellular (diagrammatic). × 13. Fig. 39. Same, enlarged view of lower portion. × 43. Fig. 40. L.s. ovule showing centripetal wall formation in endosperm (diagrammatic). × 13. Fig. 41. Magnified view of the smaller nucellar embryo (marked *ne*) and adjacent endosperm tissue from Fig. 40. × 333.

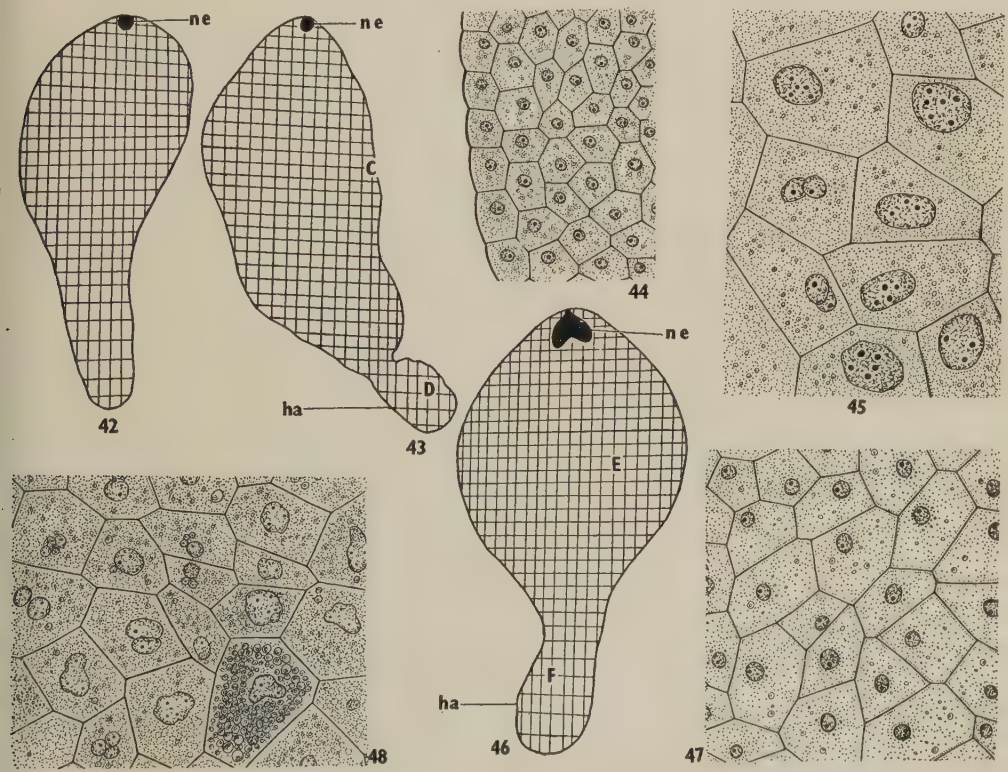
explanation may perhaps hold good for *Aegle marmelos*.

The chalazal end of the embryo sac contains dense cytoplasm and prominent nuclei which divide actively. A few short and blunt lobes are formed at the base of the embryo sac which probably function as absorbing organs and consume the adjacent tissue. Centripetal walls are initiated at the micropylar end and progress downwards (Figs. 38, 39, 41). While wall formation takes place all along the periphery of the embryo sac and around the embryo, the central portion remains occupied by a large vacuole with a few free nuclei along its margin (Figs. 40, 63, 65)[2]. Gradually it becomes wholly cellular at the late globular stage of the embryo.

The embryo sac enlarges considerably and its upper part, which forms the 'endosperm proper', becomes markedly broad, while the lower remains narrow and tubular (Figs. 42, 43, 46, 49-51). The two parts are often connected by a narrow isthmus (Figs. 40, 43).

The endosperm proper expands enormously and digests the adjoining nucellus (Figs. 51, 53). The chalazal tissue, around the embryo sac and as far down as the hypostase, also presents a famished appearance and the cell walls gradually collapse (Figs. 52, 54). This aggressive activity suggests that the tubular process plays a haustorial role. The haustorium attains its maximum activity at the heart-shaped stage of the embryo.

2. In dissections (Figs. 38, 42, 43, 46) the central vacuole is masked by the peripheral layers of cellular endosperm.



FIGS. 42-48 — (*ha*, haustorium; *ne*, nucellar embryo). All figures from dissected whole mounts. Figs. 42, 43, 46. Outline diagrams of endosperm and embryo (diagrammatic). × 13. Figs. 44, 45. Portions *C* and *D* in Fig. 43 enlarged to show the detailed structure of endosperm tissue. × 333. Figs. 47, 48. Enlargements of portions *E* and *F* marked in Fig. 46. × 333.

During subsequent development it shrinks, becomes coiled, and disorganizes (Figs. 53, 54). The degeneration starts at the upper end and gradually spreads downwards (Fig. 52).

Mauritzon (1935) also observed that in *Empleurum serrulatum*, *Coleonema album*, *Ptelea trifoliata* and *Triphasia aurantiola*, the endosperm usually extends in the form of a small tube and destroys the nucellus. In *E. serrulatum* the base of the embryo sac curves and becomes J-shaped, and in one instance he found that the nuclear endosperm had become divided by a wall into an upper smaller and a lower larger chamber.

During free nuclear divisions numerous oil globules appear in the cytoplasm (Fig. 36). At first they are uniformly distributed in the embryo sac but later there is a greater accumulation in the chalazal region (Fig. 37).

The endosperm proper consists of uninucleate cells with scanty cytoplasm and a small amount of food reserves (Figs. 44, 47). The cells of the haustorium, on the other hand, are larger, richly cytoplasmic and packed with reserve food, and many of them are multinucleate (Figs. 45, 48). Nuclear fusions giving rise to irregular polyploid nuclei are common (Figs. 45, 48).

The embryo grows at the expense of the endosperm proper and only two layers of the latter persist in the mature seed. Walls of the endosperm cells are greatly thickened (Fig. 86b).

Nucellar Embryony[3] — As mentioned earlier, in most of the embryo sacs the egg apparatus collapses and a healthy zygote could not be seen in them. By the time approximately 32 endosperm nuclei have been produced, one (sometimes two — Fig. 60; rarely more) of the nucellar cells at the micropylar end of the embryo sac becomes richly cytoplasmic (Fig. 36). In later stages it appears much more conspicuous, protrudes into the embryo sac (Figs. 37, 55-57) and divides to produce an adventive embryo.

The first division of the ' plasma-rich ' cell is usually transverse or slightly oblique (Figs. 58-60). Further divisions are irregular and result in the formation of an embryonal mass (Figs. 61-69). Occasionally, as many as three such masses may develop in the same ovule (Figs. 70-76). The nucellar embryos often exhibit varying degrees of fusion, especially in the region of the radicle (Figs. 73-76). This feature may be responsible for the appearance of seedlings with multiple shoots.

Even when several embryos develop concurrently, they are usually at different stages of development and only one of them takes the lead (Figs. 40, 70-72, 77, 78). One preparation showed an exceptionally elongated embryo (Figs. 68, 69).

The embryo passes through the globular and heart-shaped stages (Figs. 77-79) and at maturity it has two massive but often unequal cotyledons (Figs. 80, 81). They are well supplied with vascular elements and oil glands are distributed along the periphery (Figs. 80, 81). Whereas nucellar embryony is common in *Aegle marmelos*, the possibility of a zygotic embryo cannot be completely ruled out.

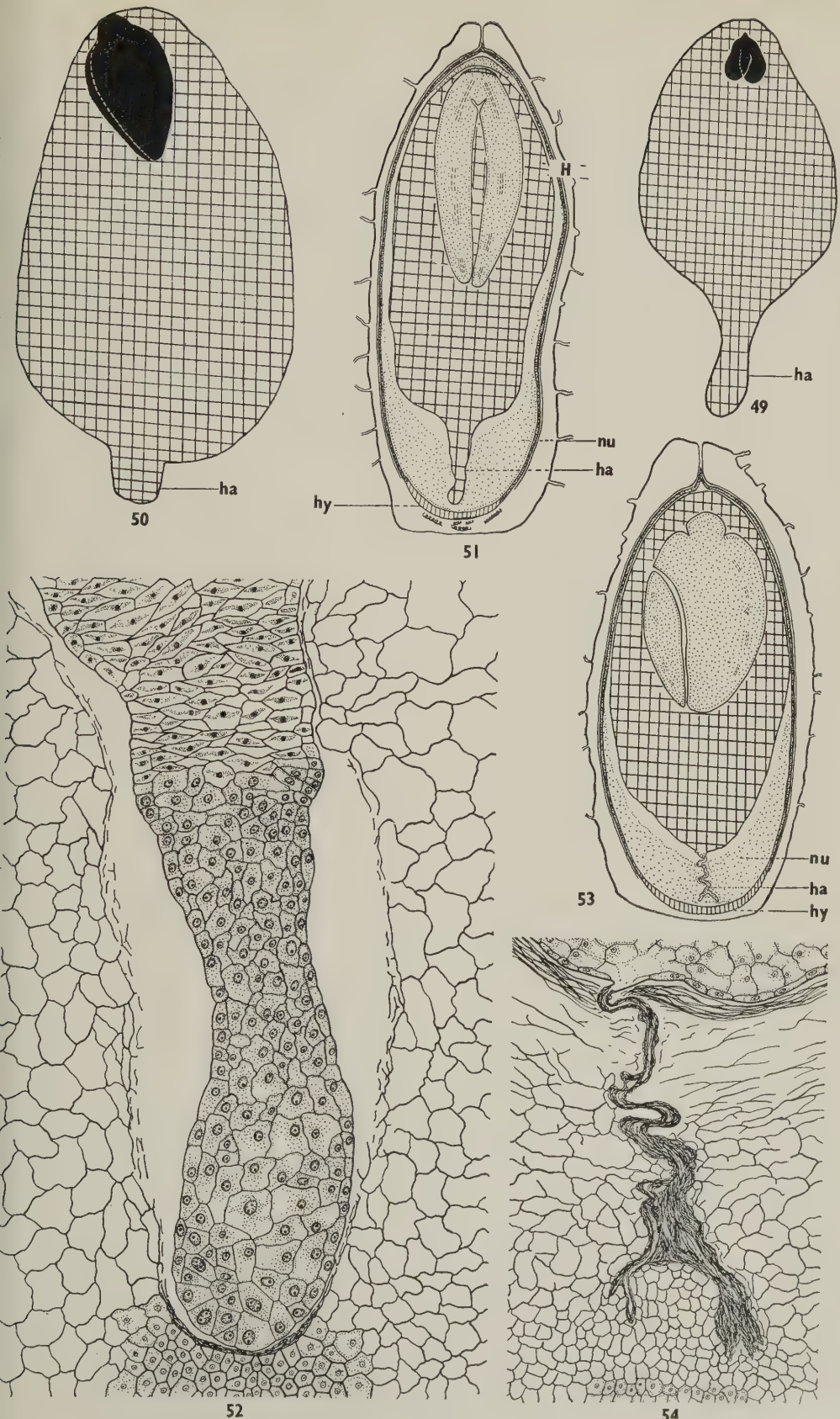
Seed Coat — Of the 3-4 layers of the inner integument (Fig. 82), the innermost becomes filled with a tanniniferous substance while the outer layers become flattened and gradually lose their identity (Figs. 83-85, 86b).

The cells of the 4-5 layered outer integument divide periclinally so that it becomes 8-12 layered (Figs. 82-84). Then the cells enlarge and become tangentially elongated (Fig. 85). During maturation of the seed coat the outer epidermis becomes heavily lignified and pitted (Fig. 86b). The hypodermis and some of the

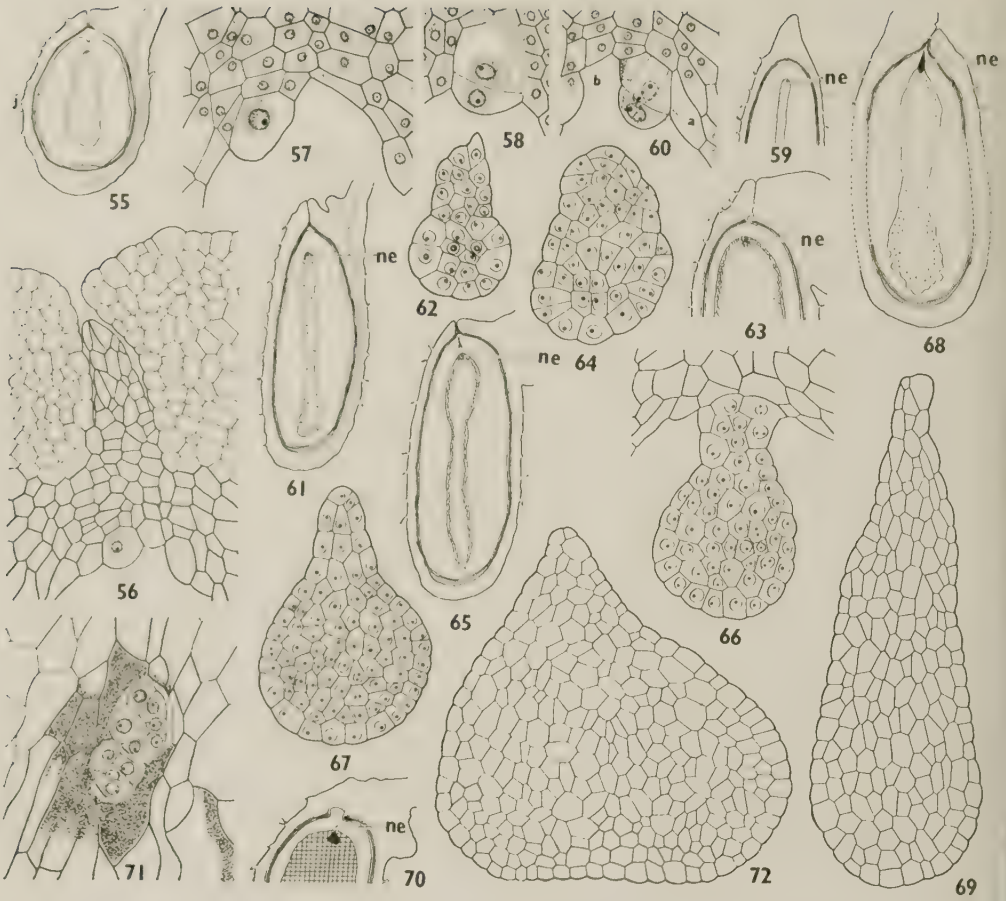
3. A brief report has already been published elsewhere (Johri & Ahuja, 1956).

→

Figs. 49-54 — (*ha*, haustorium; *hy*, hypostase; *nu*, nucellus). Figs. 49, 50. Dissected whole mounts of endosperm and embryo. × 13. Figs. 51, 53. L.s. young seeds with progressive degeneration of the chalazal endosperm haustorium (diagrammatic). × 13. Figs. 52, 54. Magnified views of the haustorium shown in Figs. 51 and 53 respectively. Fig. 52. ×150; Fig. 54. ×83.



FIGS. 49-54



FIGS. 55-72 — (*ne*, nucellar embryo). Figs. 55, 59, 61, 63, 65, 68. L.s. ovules showing position of nucellar embryos; endosperm is free nuclear in Figs. 55, 59, 61 and 68 whereas centripetal wall formation has occurred in Figs. 63 and 65 (diagrammatic). Fig. 55. ×15; Figs. 59, 61, 63, 65 and 68. × 10. Fig. 56. Magnified view of the micropylar part of nucellus from Fig. 55 showing a 'plasma-rich' cell. × 250. Fig. 57. Same as Fig. 56, but from another ovule. × 375. Fig. 58. Two-celled nucellar embryo. × 375. Fig. 60. Two-celled nucellar embryo (marked 'a') is superimposed by another enlarged nucellar cell (marked 'b'). × 375. Figs. 62, 64, 66, 67, 69. Enlargements of the nucellar embryos from Figs. 61, 63, 65 and 68 respectively; the ovule for Fig. 67 is not represented here. Figs. 62, 64, 66, 67. ×375; Fig. 69. ×250. Fig. 70. L.s. upper half of ovule with two young nucellar embryos; note another heart-shaped embryo and cellular endosperm (diagrammatic). × 10. Figs. 71, 72. Enlarged views of the three adventive embryos shown in Fig. 70. Fig. 71. × 375; Fig. 72. × 250.

innermost layers of the outer integument show the deposition of crystals. In between the crystal-layers lie a few layers of tangentially flattened cells (Fig. 86b). Thus, the seed coat is derived mainly from the outer integument and the inner epidermis of the inner integument.

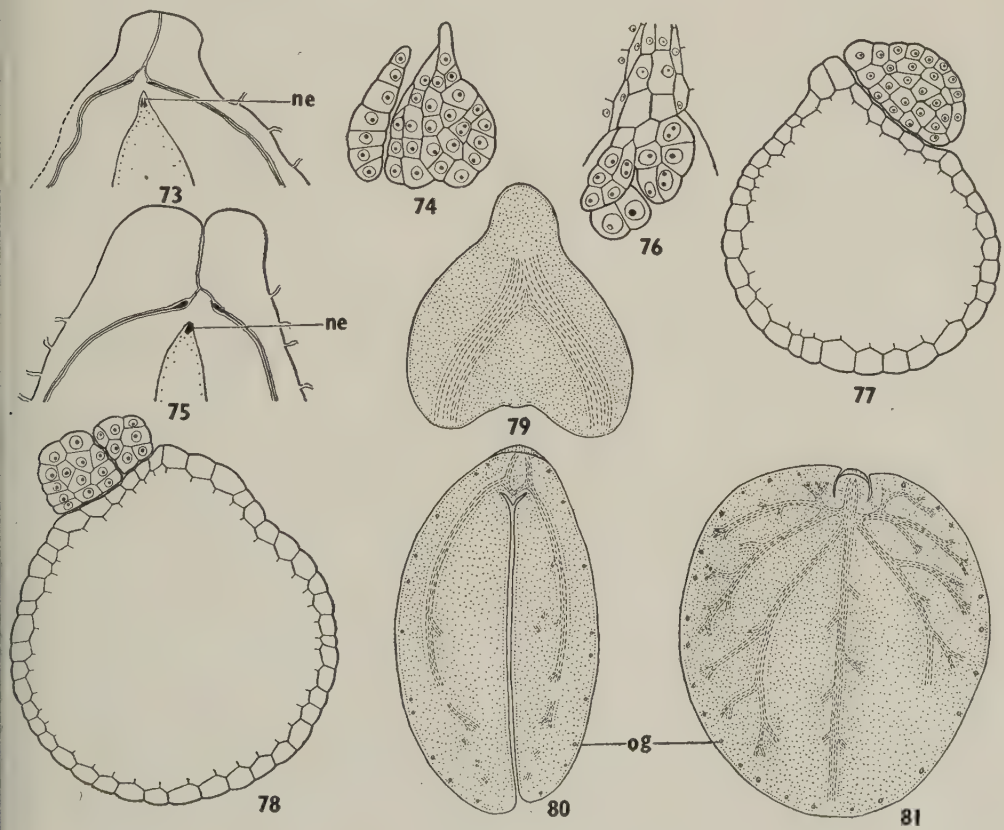
Some of the epidermal cells (Fig. 83) of the outer integument develop into multiseriate, simple or branched hairs (Figs. 84, 87) with thickly cutinized peripheral cells enclosing a few rows of lignified and pitted cells (Figs. 86b-88). These hairs form a dense covering on the seed (Fig. 86a). Multicellular hairs have also been reported in *Citrus trifoliata* (Netolitzky, 1926).

After the free nuclear endosperm has advanced considerably, the nucellar cells enlarge, become vacuolated and slightly

thick-walled. At the micropylar end they grow more rapidly on the periphery resulting in a central depression (Figs. 55, 56, 65). Soon after, the middle region resumes active growth and protrudes not only beyond the outgrown rim of the nucellus but also beyond the inner integument. It forms a more or less conical beak which fits against the outer integument (Fig. 68). Finally, however, due to the growth of the latter in the micropylar region, the nucellar beak is crushed. Mauritzon (1935) also observed more or less a similar behaviour of the nucellus in *Barosoma serratifolia* and *Empleurum serrulatum*.

The expansion of the endospem, particularly in the upper part of the ovule, crushes the nucellus and only its remnants persist in the mature seed (Fig. 86b). Netolitzky's (1926) statement that 4-5 layers persist in the seed of *Aegle marmelos* could not be confirmed by us.

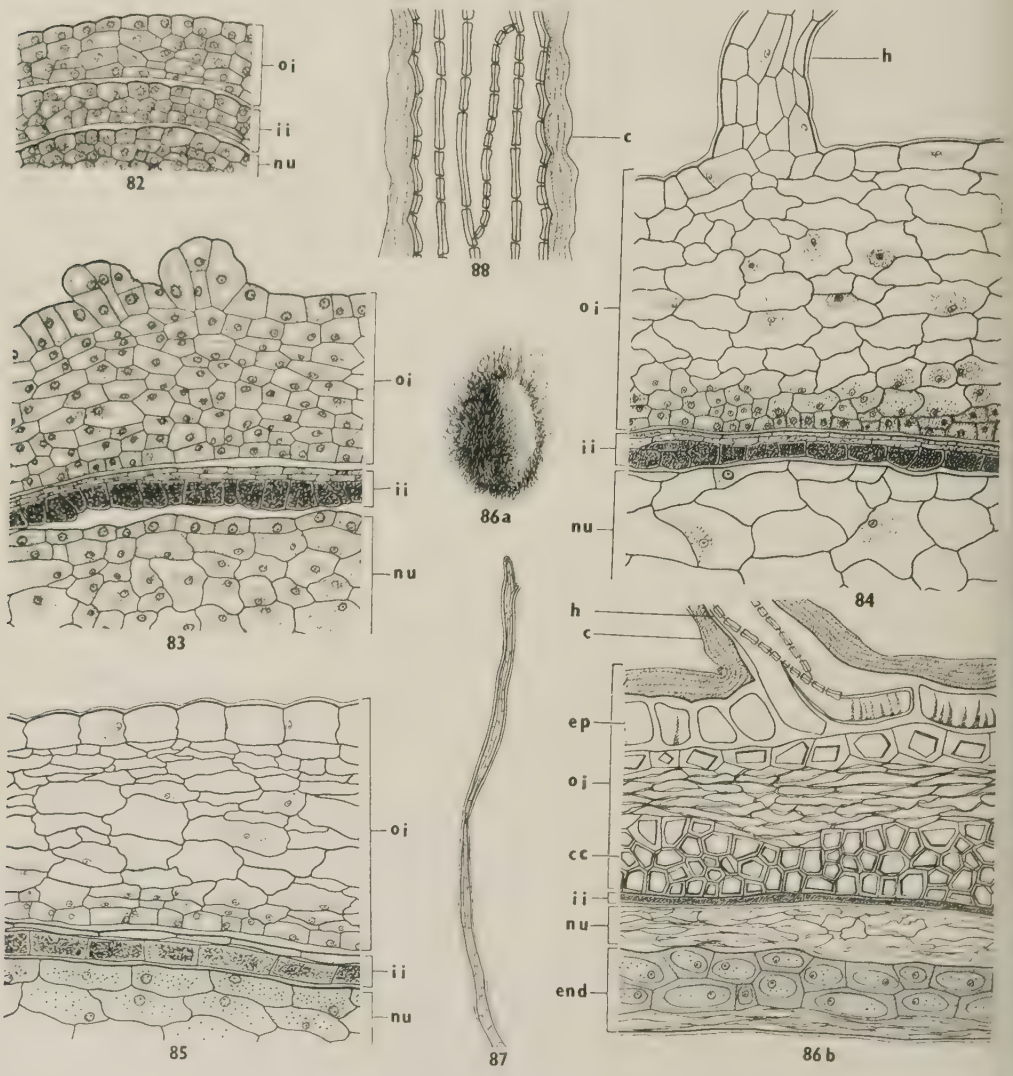
The seed is oval or oblong and somewhat compressed. It looks white when young but turns yellow on ripening and is surrounded by a very tenacious, slimy, transparent mucilage. There is a high degree of sterility and out of a total of about 200 ovules in an ovary, only 50-60 seeds are formed.



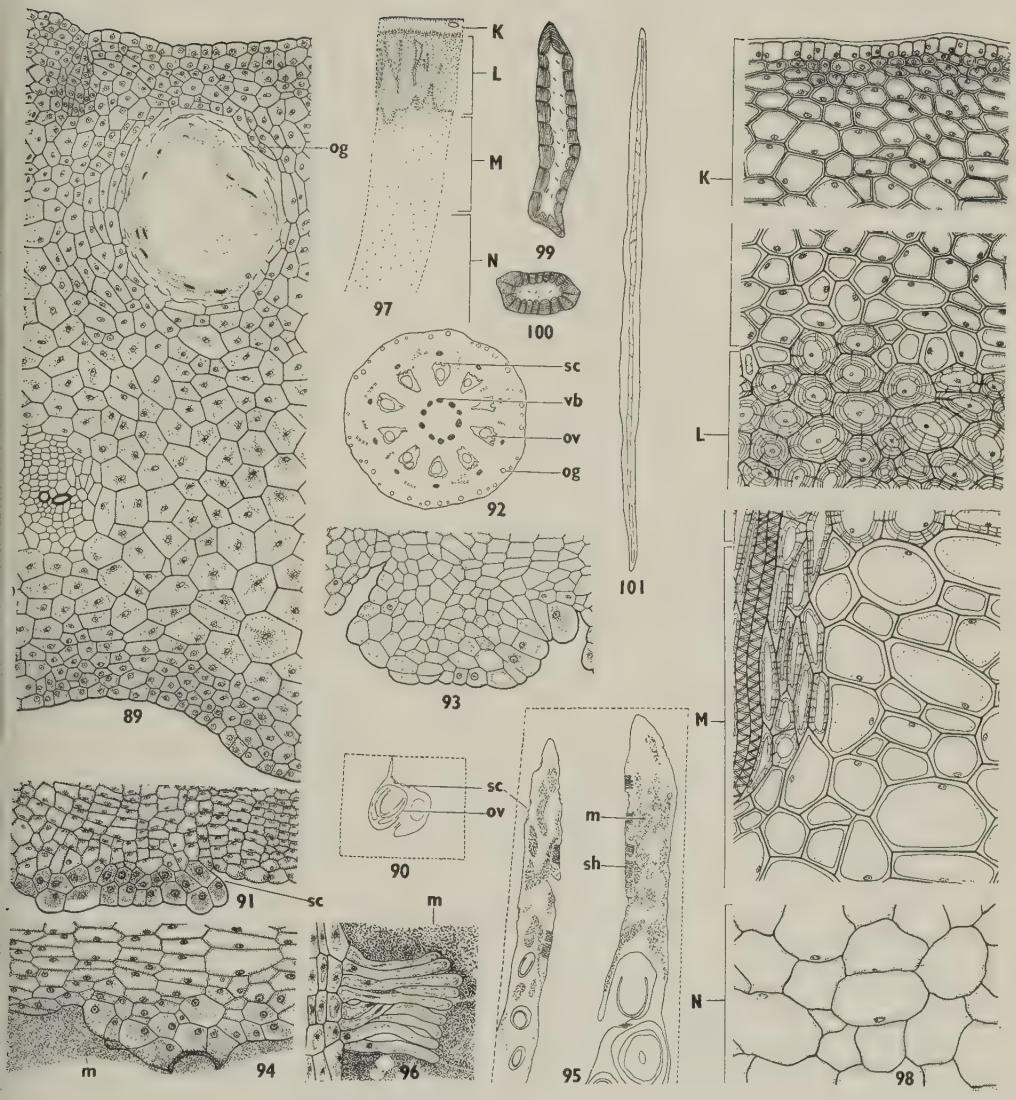
FIGS. 73-81 — (*ne*, nucellar embryo; *og*, oil gland). Figs. 73, 75. L.s. upper part of ovules showing nucellar embryos at micropylar end (diagrammatic). ×15. Figs. 74, 76. Enlargements of adventive embryos from Figs. 73 and 75 respectively. × 365. Figs. 77, 78. Whole mounts of adventive embryos. × 250. Fig. 79. Heart-shaped embryo. ×112. Figs. 80, 81. Mature embryos in l.s. and surface view. × 10.

Pericarp — At the mature embryo sac stage the pericarp consists of 30-35 layers of parenchymatous tissue (Fig. 89). The cells of the 4-6 hypodermal layers, on either side, are smaller and have denser contents. The middle region consists of larger and vacuolated cells. The pericarp is well supplied with vascular traces and numerous oil-ducts occur in the peripheral region (Fig. 92).

Even before the formation of the endosperm, meristematic activity in a few layers next to the inner epidermis results in an increased thickness of the pericarp (Figs. 91, 94). At places the cells of the inner epidermis and 1-2 adjoining layers are



Figs. 82-88 — (*c*, cuticle; *cc*, crystal cells; *end*, endosperm; *ep*, epidermis; *h*, hair; *ii*, inner integument; *nu*, nucellus; *oi*, outer integument). Figs. 82-85. Enlarged portions of integuments and nucellus marked *B*, *G*, *H* and *J* in Figs. 17, 40, 51 and 55 respectively. × 333. Fig. 86a. Seed with the surface coat of hairs. × 1. Fig. 86b. Portion of testa (l.s.) from mature seed. × 333. Fig. 87. Hair from testa (whole mount). × 60. Fig. 88. Same, a portion magnified. × 333.



FIGS. 89-101 — (*m*, mucilage; *og*, oil gland; *ov*, ovule; *sc*, secretory cells; *sh*, secretory hairs; *vb*, vascular bundle). Fig. 89. L.s. portion of pericarp at mature embryo sac stage. × 200. Fig. 90. Outline diagram for Fig. 91. × 20. Fig. 91. Enlarged view of secretory cells. × 200. Fig. 92 T.s. ovary showing groups of secretory cells projecting into the locules (diagrammatic). × 14. Fig. 93. A group of secretory cells enlarged from Fig. 92. × 200. Fig. 94. Same as Figs. 91 and 93, but advanced stage, densely dotted area represents mucilage. × 200. Fig. 95. L.s. portion of ovary showing the locules filled with mucilage, secretory hairs are also present on the ovarian wall (diagrammatic). × 12. Fig. 96. Magnified view of secretory hairs. × 200. Fig. 97. L.s. pericarp at the late globular stage of embryo (diagrammatic). × 12. Fig. 98. Enlargements of portions marked *K*, *L*, *M*, and *N* in Fig. 97. × 200. Figs. 99-101. Sclereids and fibre from macerated preparation of rind. × 200.

richly cytoplasmic and contain larger nuclei (Figs. 90, 91). Groups of these cells bulge into the locules of the ovary and form mucilage-secreting glands (Figs. 92, 93). Mucilage is also secreted by the unicellular glandular hairs, present on the

wall of the locule and on the funiculus (Figs. 95, 96). It fills up the whole cavity in which the seeds remain embedded.

At the globular stage of the proembryo, the pericarp becomes distinguishable into four zones (Fig. 97). An outer region of 16-18 layers of thick-walled cells (Fig. 98, *K*) is followed by about 35 layers consisting of stone cells and fibres (Figs. 98, *L*; 99-101). Then there is another zone of 13-15 thick-walled layers but here the cells are much larger (Fig. 98, *M*). These three zones occupy about one-third the thickness of the rind. The innermost and most extensive region comprises parenchymatous cells (Fig. 98, *N*) which form the yellowish sugary pulp.

Discussion

Chakravarthy (1936) reported that the ripe seeds of *Aegle marmelos* did not show a single case of polyembryony. He states: " But on examining serially sectioned preparations of a large number of young seeds, two cases of nucellar embryos were noticed. In both these cases the nucellar embryo is extremely small when compared with the normal egg-embryo by its side . . . Nucellar embryos are very rarely found in *Aegle* and even then they seem to stop development at a very early stage . . . " Chakravarthy also observed nucellar polyembryony in *Murraya koenigii* (1935) and *M. exotica* (1936).

As mentioned earlier, in *A. marmelos* three-fourths of the ovules in an ovary contain disorganized gametophytes. In most of the remaining ovules degeneration sets in at the mature embryo sac stage, the antipodals degenerating first and then the egg apparatus. However, the polar nuclei remain healthy and sometimes even the egg. Several embryo sacs showed remnants of pollen tubes but none of them gave any indication of the development of a sexual embryo. However, initiation and development of nucellar embryos was quite common. Chakravarthy's report that the latter are very rare is, therefore, not confirmed.

In some other genera of the Rutaceae, both zygotic and nucellar embryos are formed. Strasburger (1878) and Osawa (1912) found that in *Citrus aurantium* some of the nucellar cells differ in size and contents from the neighbouring cells. They become rounded and enlarged and show granular contents. Each produces an embryonic mass which gradually finds its way into the cavity of the embryo sac and develops further. Ordinarily, in an embryo sac there is a normal zygotic embryo with two or more adventive embryos.

In *C. paradisi* and *C. aurantium*, besides nucellar embryony, Bacchi (1943) indicates the possibility of polyembryony due to the production of zygotic embryos from multiple gametophytes. Multiple gametophytes also occur in *C. grandis* (Banerji, 1954) but only a single zygotic embryo is formed and adventive embryos are absent. Judging from the examination of some seedlings of *Citrus*, Frost (1926, 1938) proposed the hypothesis of cleavage polyembryony to explain the production of two hybrid seedlings from a single seed. However, no one has so far observed the cleavage of zygotic embryo in this genus.

Polyembryony in *Citrus* and related genera (*Poncirus* and *Fortunella*) has also been investigated by H. J. Webber (1900), Torres (1936), Traub (1936) and Śokolśkaja (1938) (*see also* J. M. Webber, 1940; Maheshwari & Sachar, 1957).

Mauritzon (1935) could not decide whether in *Empleurum serrulatum* and *Dictamnus albus* the single embryo present in the embryo sac was of zygotic or nucellar origin. On the other hand, both types of embryos were common in *Ptelea trifoliata*, *Xanthoxylum bungei* and *Triphasia aurantiola*. In the last two species the nucellar embryos arise from deep-seated cells. In *T. aurantiola* he observed a case of five adventive embryos originating from the cells of the nucellar cap (derivatives of the nucellar epidermis). A rather unusual condition is reported in *Phellodendron sachalinense* where the embryo (whether it was nucellar or zygotic is not known) showed several free nuclei without any walls between them. Cappalletti (1930) also observed a similar embryo in *Ruta patavina*.

Further comparative work is necessary on the phenomenon of adventive

embryony in both wild and cultivated members of the Rutaceae not only from the developmental standpoint but also with a view to see whether the initiation of adventive embryos is dependent on pollination and endosperm formation.

Summary

Aegle marmelos is a moderate-sized tree with much branched cymose inflorescences bearing tetra- or penta-merous flowers. The multilocular ovary has two rows of ovules on axile placentae.

The anthers are dithecous and the wall consists of the persistent epidermis, fibrous endothecium, three middle layers and the glandular, multinucleate tapetum. The young anthers are clothed with epidermal, 1-celled hairs which fall off at maturity.

The reduction divisions are simultaneous and both tetrahedral and decussate tetrads of microspores are formed. Only 2-celled pollen grains were observed. They show 60-70 per cent germination in 40 per cent sucrose and 1 per cent agar.

The bitegmic and crassinucellate ovule is anatropous. The hypodermal archesporium is multicelled but only one of the cells functions giving rise to a linear, sometimes T-shaped, tetrad. The embryo sac conforms to the Polygonum type.

Approximately 75-80 per cent of the mature embryo sacs degenerate completely. In most of the remaining ones also the antipodal cells disorganize first and then the egg apparatus. However, the polar nuclei remain healthy and in some cases also the egg.

The endosperm is Nuclear. Wall formation is centripetal and is initiated at the micropylar end gradually progressing downwards. The chalazal end grows into an aggressive tubular haustorium which sometimes becomes coiled up. It shrinks and degenerates during the maturation of seed.

Nucellar embryony is the rule, and although several adventive embryos grow concurrently, but only one of them attains maturity.

The seed coat consists of the thick-walled outer epidermis of the outer integument, the tannin-filled inner epidermis of the inner integument and the remnants of the intervening 8-10 layers some of which contain crystals.

The seed coat is thin and bears thick-walled hairs. There are two layers of endosperm, and a massive dicotyledonous embryo. The reserve food in the endosperm and embryo is fatty. Only 50-60 seeds mature in an ovary containing nearly 200 ovules.

The pericarp consists of the hard outer rind, and a broad inner zone of parenchymatous cells which are rich in sugar and form the pulp.

From the inner margins of the pericarp develop mucilage-secreting glands which grow into the locule. The glandular hairs on the funiculus also secrete mucilage.

We are indebted to Prof. P. Maheshwari for going through the manuscript and for allowing a free use of his personal library. Thanks are also due to Mr R. N. Chopra and Mr S. P. Bhatnagar for the assistance in the preparation of this paper.

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MORPHOLOGICAL AND EMBRYOLOGICAL STUDIES IN THE FAMILY SANTALACEAE. I—*COMANDRA UMBELLATA** [L.] NUTT.

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The floral structure and embryology of the family Santalaceae are very peculiar in many respects. The flowers are monochlamydeous and are borne in racemes or in branched cymes. Sometimes, the flowers are borne in the axils of bracts which may be leafy or scale-like (Pilger, 1935). All gradations from hermaphrodite to staminate or pistillate flowers are met with.

Most systematists consider the ovary to be unilocular, but Schulle (1933), Rutishauser (1937), Schaeppi (1942), Smith & Smith (1942), Rao (1942) and Paliwal (1956) state that at least in some genera (e.g. *Thesium*, *Osyris*, *Santalum*, *Leptomeria* and *Choretrum*) it is chambered at the base, each chamber corresponding to an ovule.

The ovules are borne on a central placentum which may be straight or spirally twisted. In *Santalum* and *Thesium* the ovules are merely small outgrowths with the tips pointing towards the base of the ovary, whereas in *Osyris* and *Scleropyrum* they curve upwards and

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become anatropous. Opposing views have been expressed regarding the nature of the ovules. Engler & Prantl (1889), and Iyengar (1937) regard them as naked, while Warming (1878), Schulle (1933), Goebel (1933), Rutishauser (1937) and Paliwal (1956) consider them to be tegmic. The embryo sac conforms to the Polygonum type and the mature gametophyte sends out a chalazal caecum which enters into the placental column. In *Santalum* the tip of the embryo sac grows beyond the ovule into the ovarian cavity and reaches slightly below the placental tip.

In later stages the chalazal caecum functions as the endosperm haustorium. The endosperm may be Helobial (*Santalum* — Rao, 1942; Paliwal, 1956) or Cellular (*Thesium, Osyris* — Schulle, 1933; Rutishauser, 1937; Rao, 1942; Paliwal, 1956). Paliwal (1956) has recently reported a micropylar haustorium in *Santalum* and secondary haustorial cells in *Thesium*. Another feature of interest in the former is the fusion of the endosperms of different embryo sacs in an ovary resulting in a composite mass comparable to that of the Loranthoideae.

The present paper deals with the floral morphology and embryology of *Comandra umbellata*, a North American genus of the Santalaceae.

Material and Methods

At the request of Professor P. Maheshwari, the material of *Comandra umbellata* was very kindly collected and sent to me by Professors F. H. Smith (Oregon, U.S.A.), H. F. Copeland (Sacramento, U.S.A.), H. F. Totten (North Carolina, U.S.A.) and J. T. Howell (California, U.S.A.). The customary methods were followed for preparing the material for microtomy. Sections were cut 5-20 microns thick and stained with safranin-fast green.

Since microtome sections alone did not permit a clear understanding of the embryo sac and the endosperm haustoria, dissections and whole mounts were studied in most cases. For this purpose the material was treated with 5 per cent KOH at 40°C for 24 hours, thoroughly washed with water and the embryo sacs and endosperms were dissected out under a stereoscopic binocular. These were then stained with Delafield's haematoxylin and mounted in a mixture of the stain and Zirkle's medium (Johansen, 1940). This medium has the advantage that it holds the coverslip firmly and there is no need to seal it with canada balsam.

Investigation

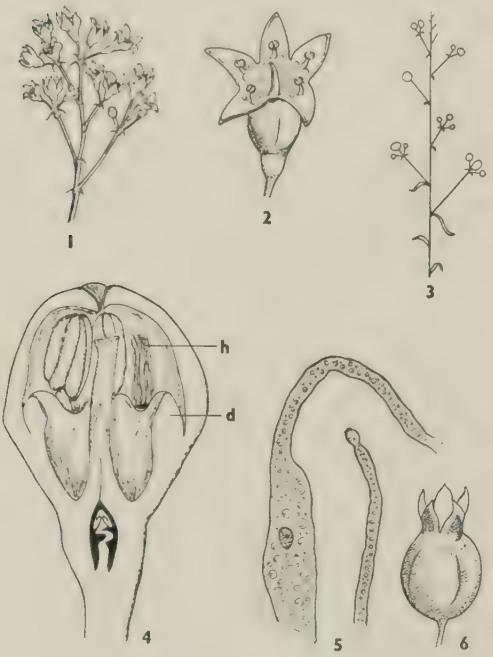
Floral Morphology — The inflorescence consists of simple cymes arranged in a racemose fashion. Each cyme arises in the axil of a bract and is three-flowered (Fig. 1), the lateral flowers lying in the axils of prophylls. The cyme may often undergo reduction by suppression of one or rarely both the lateral flowers (*see also* Holm, 1924). Occasionally, the central one may fail to develop (Fig. 3). The cream-coloured, pedicellate flowers are actinomorphic and hermaphrodite.

The flower is typically pentamerous (Fig. 2) but four (Fig. 19) and six (Fig. 17) members are also not uncommon. The perianth is tubular at the base. The lobes remain united in the bud condition due to the interlocking of marginal epidermal cells. A cross-section shows that the perianth consists of parenchymatous cells with stomata on the outer epidermis. The cells of the inner epidermis become radially elongated. Some tannin-filled cells are present here and there in the mesophyll (Fig. 9). The epidermal cells at the base of the perianth, behind each stamen, produce numerous unicellular hairs (Fig. 4). These have a conspicuously broad base containing the nucleus and a constricted upper part, and are packed with oil globules (Fig. 5). In older flowers they loose their contents and shrivel up. Similar hairs are also found in *Thesium* and *Santalum*. According to Van Tieghem (1869), the hairs are subepidermal in origin. Ewart (1892) does not state whether they are epidermal or sub-epidermal but her figures indicate that they develop from the epidermis. Schulle (1933) and Rao (1942) have confirmed the epidermal origin of the hairs and my observations are in agreement with theirs.

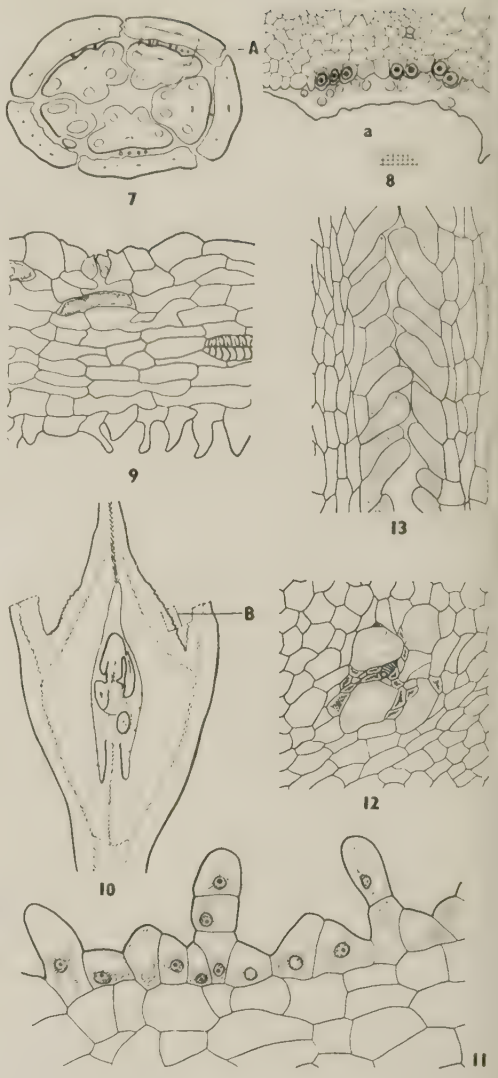
The stamens are situated opposite to the perianth lobes and have long filaments (Fig. 2) each bearing a dorsifixed introrse anther. The anther lobes are divergent at the base (Figs. 4, 21) and dehisce longitudinally. A 5-lobed disc develops between the androecium and the gynaecium and is partly fused with the perianth tube (Fig. 4).

The semi-inferior ovary is unilocular and contains two to four ovules borne on a free central, twisted placental column (Fig. 4). The style is long and hollow and the canal is lined by upwardly directed papillate epidermal cells (Fig. 13).

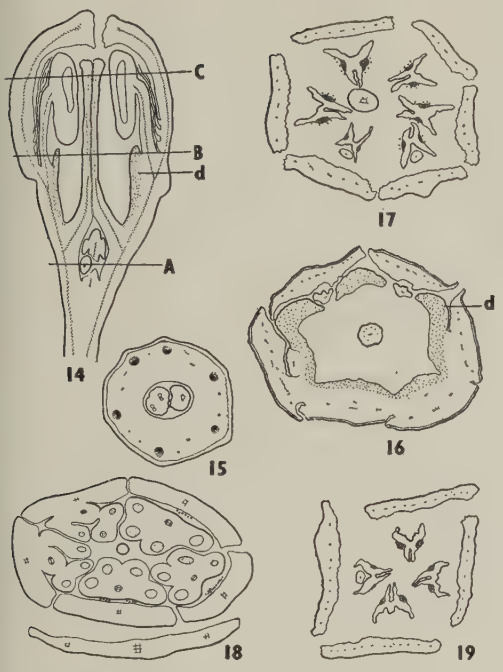
Stomata are present on the outer as well as the inner epidermis of the ovary. The epidermal cells of the upper part of the ovary also give rise to multicellular hairs (Figs. 10, 11). Tannin is present in the hypodermal layer of the receptacle and perianth, in the epidermis of the style and the connective of stamens. Sphaeraphides are common in different parts of the flower. The disposition of the floral organs in longitudinal and transverse sections is shown in Figs. 14-17.



Figs. 1-6 — Floral Morphology (*d*, disc; *h*, hair). Fig. 1. Flowering twig. × 2·5. Fig. 2. Single flower. × 4. Fig. 3. Diagrammatic representation of the inflorescence. Fig. 4. L.s. bud. × 7. Fig. 5. Enlargements of basal and apical portions of a hair. × 131. Fig. 6. Fruit with persistent perianth. × 4.



Figs. 7-13 — Floral Morphology. Fig. 7. T.s. bud. × 48. Fig. 8. Region marked *A* in Fig. 254; the shaded cells represent sections of hairs. × 222. Fig. 9. Portion of perianth lobe (l.s.). × 222. Fig. 10. L.s. ovary at mature embryo sac stage. × 23. Fig. 11. Portion marked *B* in Fig. 10. × 222. Fig. 12. T.s. placental column showing four embryo sac haustoria. × 222. Fig. 13. Part of stylar canal (l.s.); the elongated epidermal cells are obliquely oriented. × 222.



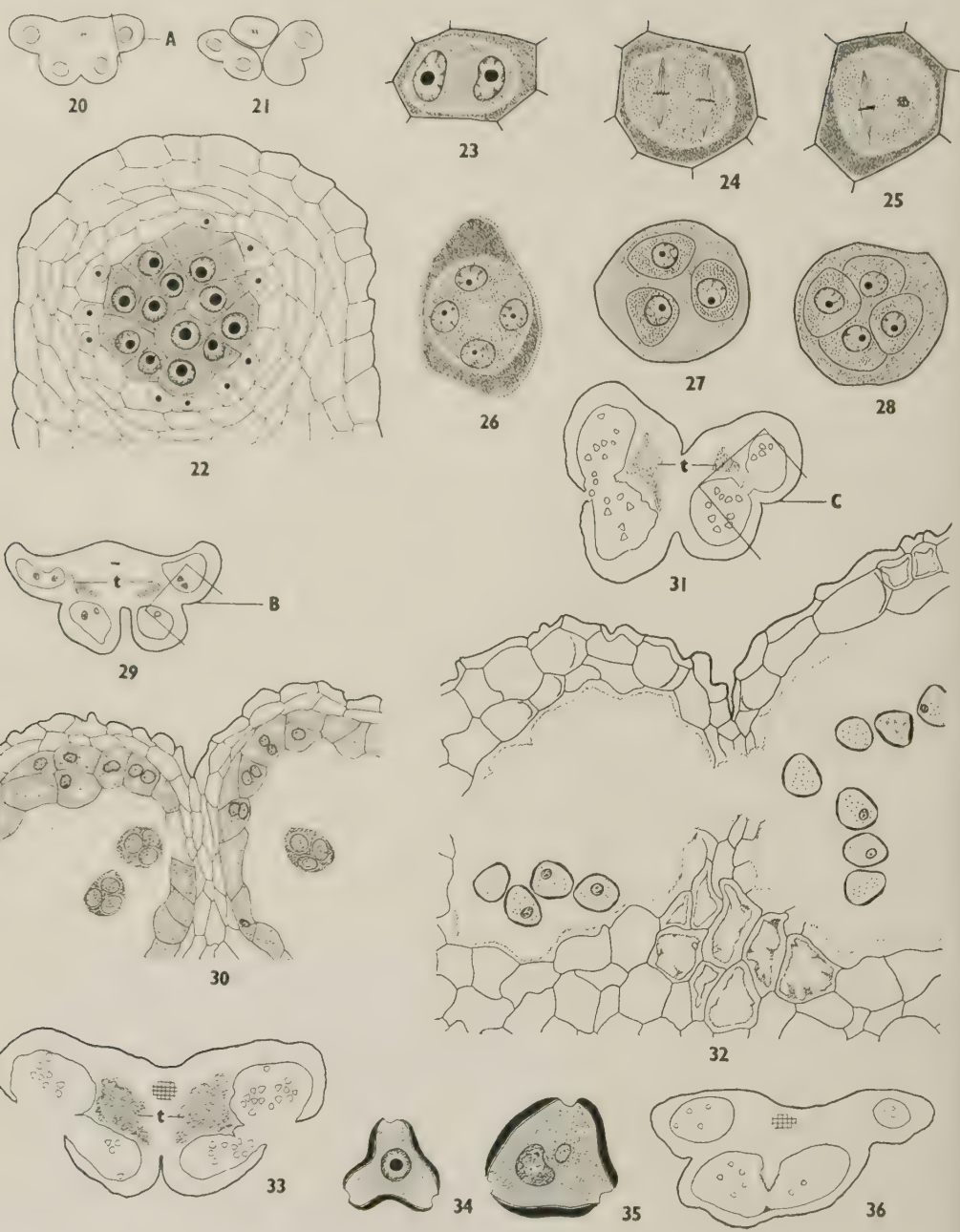
FIGS. 14-19 — Floral Morphology. (*d*, disc). Fig. 14. L.s. bud. × 31. Figs. 15-17. T.s. old flower, approximately at levels marked *A*, *B* and *C* in Fig. 14. × 15. Fig. 18. T.s. 5-lobed flower. × 35. Fig. 19. Same, 4-lobed. × 15.

MICROSPOROGENESIS AND MALE GAMETOPHYTE — The youngest anther available showed the microspore mother cells surrounded by the glandular tapetum, two to three middle layers, the endothecium and the epidermis (Figs. 20, 22). The reduction divisions are simultaneous (Figs. 23-26) and cytokinesis occurs by furrowing. During Meiosis II the spindles may lie parallel or at right angles to each other (Figs. 24, 25), resulting in decussate or tetrahedral tetrads (Figs. 27, 28). The pollen grains are triangular and have a smooth exine and a thin intine (Figs. 34, 35). They are shed at the two-celled stage and are packed with starch grains which stain deep red with safranin and often mask the nuclei.

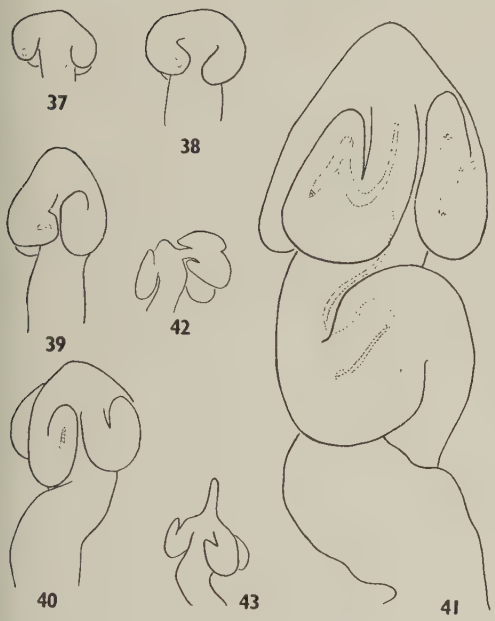
As the anther enlarges, the epidermal cells get stretched and flattened and the radially elongated cells of the endothecium acquire feebly developed fibrous thickenings (Fig. 32). The middle layers collapse by the time tetrads are formed. The tapetal cells are at first uninucleate but later become binucleate. Sometimes, the nuclear division may be followed by wall formation giving rise to a two-layered condition at places (Figs. 29, 30). The tapetum is absorbed during the enlargement of pollen grains. Finally, a dissolution of the septum between the pollen sacs brings about the confluence of the adjacent locules (Figs. 31, 32). Dehiscence occurs due to the disorganization of the narrow and thin-walled cells at the junction of the sacs (Fig. 33). In one case two pollen sacs of adjacent anther lobes had fused (Fig. 36).

OVULE — The ovules are initiated as small lateral protuberances from the apical end of the thick placental column (Fig. 37) and are directed towards the base of the ovarian cavity. With the differentiation of the megaspore mother cells they curve through 180 degrees so that the micropyles face the style (Figs. 38-40). The ovules and the placental column enlarge appreciably. The latter elongates and becomes spirally twisted (Figs. 40, 41). In one instance the tip of the placental column had elongated in the form of a pointed tip (Fig. 43), and in another, two ovules showed a common funiculus (Fig. 42).

During earlier stages, the nucellar cells cannot be clearly identified from those of the integument (Fig. 44). However, periclinal divisions in the cells surrounding the apical portion demarcate the integument (Fig. 45) which appears like a rim at the megaspore mother cell stage. Further periclinal and anticlinal divisions make the integument more massive and a narrow micropyle also becomes distinguishable (Fig. 48). The nucellus is represented by only a few epidermal cells (Fig. 45) and in this respect it resembles *Thesium* (Paliwal, 1956). The nucellar cells are completely consumed during the development of the female gametophyte (Fig. 48) so that at the 4-nucleate stage the tip of the embryo sac is in direct contact with the micropyle. The integumentary tissue is progressively absorbed by the developing endosperm. In Fig. 60 the cells at the micropylar end are still healthy but those on the sides have mostly been consumed. Subsequently, even



FIGS. 20-36 — Microsporogenesis and Male Gametophyte. Figs. 20, 21. T.s. young anther at different levels. × 65. Fig. 22. Anther lobe marked *A* in Fig. 21. × 614. Figs. 23-26. Dyad and Meiosis II. × 920. Figs. 27, 28. Tetrahedral and decussate tetrads. × 963. Figs. 29, 31. T.s. anthers at tetrad and 1-celled pollen grain stages. × 65. Figs. 30, 32. Portions marked *B* and *C* in Figs. 29 and 31 respectively. × 321. Fig. 33. Dehisced anther. × 65. Figs. 34, 35. One and 2-celled pollen grains. × 860. Fig. 36. T.s. abnormal anther. × 143.



FIGS. 37-43 — Ovule. (All figures are from whole mounts.) Figs. 37-39. Placental column with ovules at archesporium and mother cell stages. × 253. Figs. 40, 41. Same, at tetrad and mature embryo sac stages; in Fig. 41 the dotted outline represents the extension of the embryo sac in the placental column. × 253. Figs. 42, 43. Abnormal placental columns. × 26.

these cells are absorbed and the endosperm lies free in the ovarian cavity (Fig. 76).

MEGASPOROGENESIS AND FEMALE GAMETOPHYTE — In a young ovule two or three densely cytoplasmic hypodermal archesporial cells appear and show prominent nuclei (Fig. 44). One of these elongates and directly functions as the megaspore mother cell (Fig. 45). It undergoes the usual reduction divisions forming a linear tetrad of megaspores of which the chalazal one functions (Fig. 46).

The functioning megaspore enlarges, becomes vacuolate and produces the 2- (Fig. 47), 4- (Fig. 48) and 8-nucleate gametophytes in the usual way. The organized embryo sac has a characteristic shape with a broad micropylar and a narrow chalazal end (Fig. 40). The synergids are hooked and show a filiform apparatus (Fig. 51), the two polar nuclei lie adpressed to each other in the upper part, and the antipodal cells may be variously disposed (Figs. 50, 51). Sometimes only two antipodal cells are formed (Fig. 49).

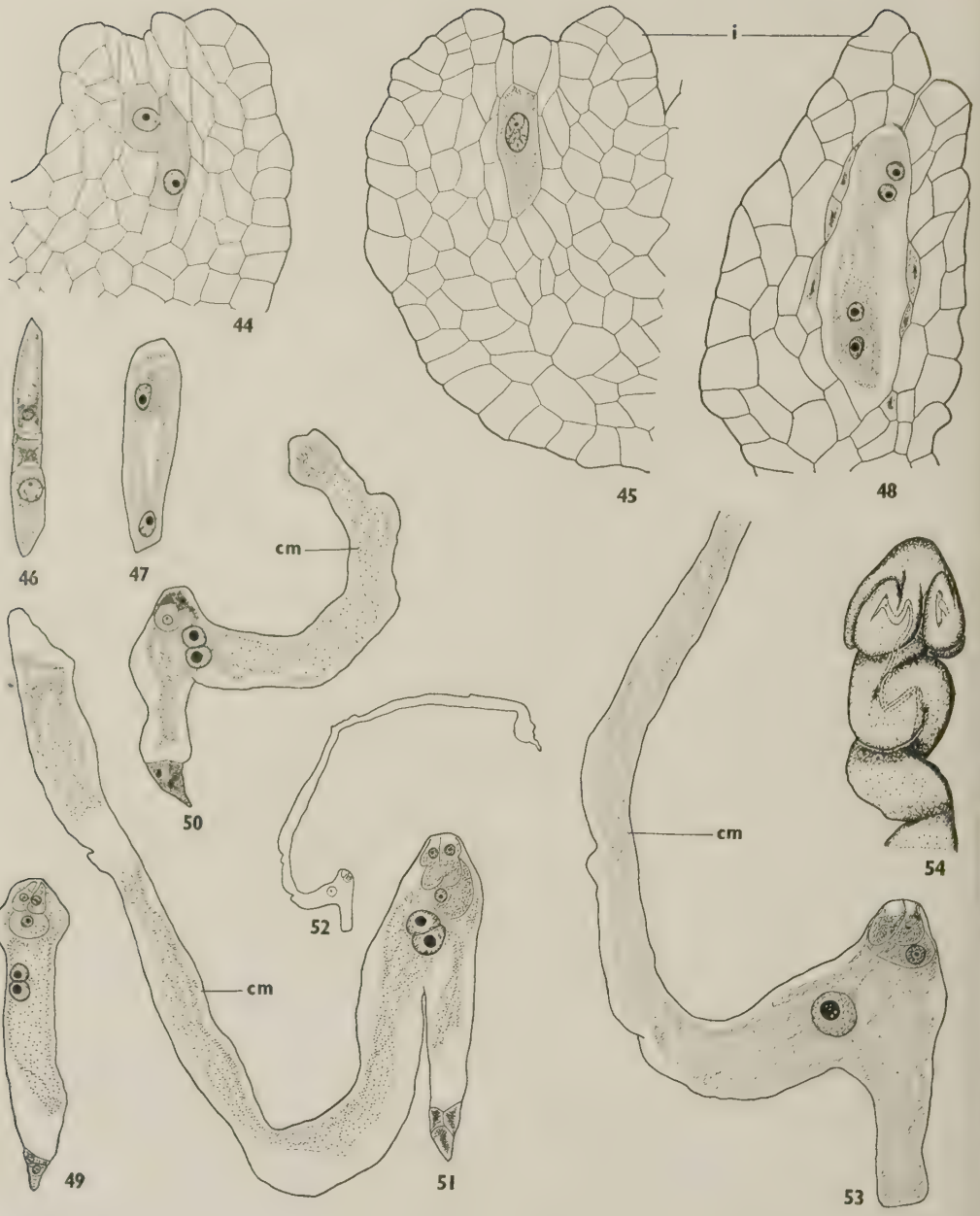
Even before fertilization the synergids and the antipodal cells degenerate. At the same time a lateral caecum arises, on the funicular side, just below the level of the egg apparatus (Fig. 50). It grows through the ovule, enters into the placenta and extends along the vascular strand (Figs. 52, 53). Thus in a cross-section of the placental column, two to four caeca surround the vascular strand (Fig. 12).

A single embryo sac develops in each ovule and by the time it reaches maturity, most of the ovular tissue is consumed.

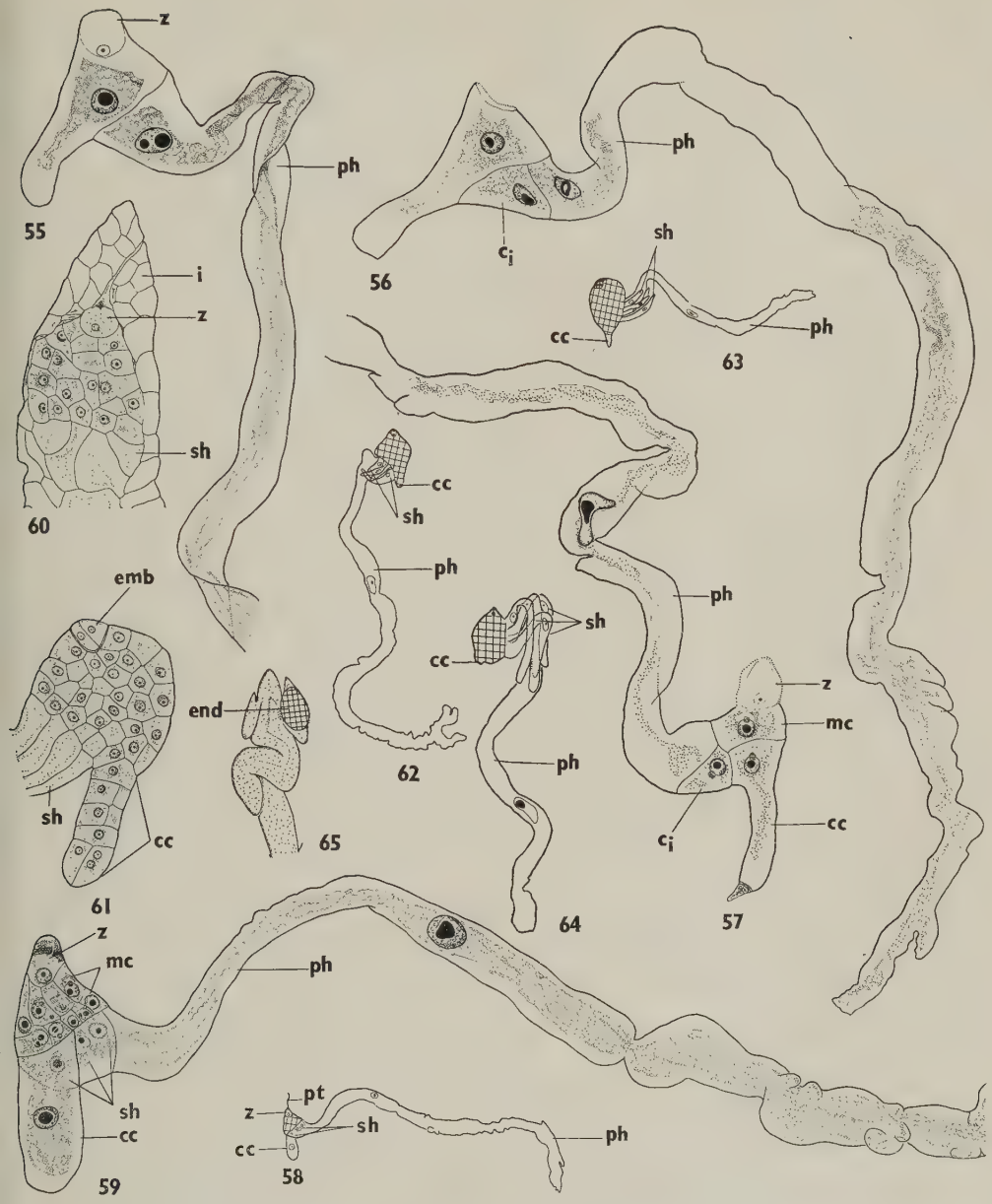
ENDOSPERM — The first division of the primary endosperm nucleus is followed by the formation of a wall at the junction of the lateral caecum and the embryo sac proper (Fig. 55). The nucleus of the caecum divides once again cutting off a small cell c_i (Fig. 56). The rest of the caecum functions as the primary endosperm haustorium. The cell c_i divides again giving rise to four cells (Figs. 57, 58) which elongate and grow out in the same direction as the primary endosperm haustorium. These cells function as secondary haustoria (Figs. 59-64) and appear as large vacuolated structures in sections (Fig. 61). In the mean time, the embryo sac gets divided into a large chalazal and a small micropylar chamber (Fig. 57). The former either degenerates as such (Figs. 59, 63), or may produce a few cells (Fig. 61) which get incorporated in the endosperm proper (Figs. 61, 62). The latter is derived mostly from the activity of the micropylar chamber (Figs. 58-64).

The primary haustorium, which is richly cytoplasmic, possesses a large hypertrophied nucleus, develops peripheral outgrowths and may sometimes even branch (Fig. 62). The primary and secondary haustoria remain healthy for a long time and their remnants can be recognized even at the dicotyledonous stage of the embryo.

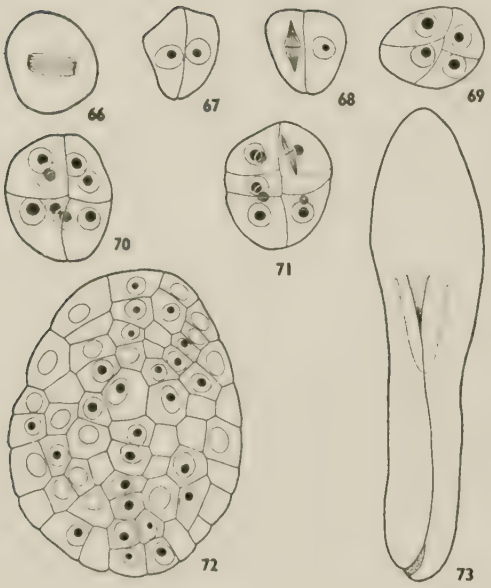
The endosperm first consumes the lateral integumentary tissue (Fig. 60) and then the apical cells. The placental column and the parenchymatous endocarp are used up soon after (Figs. 78, 79).



FIGS. 44-54 — Megasporogenesis and Female Gametophyte. (Figs. 50-54 are from whole mounts, rest from microtome sections. *i*, integument; *cm*, caecum.) Fig. 44. L.s. ovule with two archesporial cells. × 575. Fig. 45. Same, showing mother cell. × 575. Fig. 46. Linear tetrad of megaspores. × 575. Fig. 47. Two-nucleate embryo sac. × 575. Fig. 48. L.s. ovule with 4 nucleate embryo sac. × 575. Fig. 49. Organized gametophyte. × 254. Figs. 50, 51, 53. Stages in development of the lateral caecum. × 254. Fig. 52. is the outline diagram of Fig. 53. × 59. Fig. 54. Placental column; dotted line indicates the extension of the embryo sac. × 8.



FIGS. 55-65 — Endosperm. (Figs. 60 and 61 from microtome sections, rest are from whole mounts. *cc*, chalazal chamber; c_i, small cell; *emb*, embryo; *end*, endosperm; *i*, integument; *mc*, micropylar chamber; *ph*, primary haustorium; *pt*, pollen tube; *sh*, secondary haustoria; *z*, zygote.) Fig. 55. Two-celled endosperm with the lateral primary haustorium. × 198. Fig. 56. Three-celled endosperm; a small cell (c_i) has been cut off in the caecum. × 198. Fig. 57. Four-celled endosperm, the chalazal as well as the micropylar chambers have been delimited. × 198. Fig. 58. Outline diagram of Fig. 59. × 35. Fig. 59. Same, enlarged; the micropylar chamber has produced two tiers of cells. The small cell (marked c_i in Fig. 56) has given rise to initials of the secondary haustoria (*sh*): note the undivided chalazal chamber (*cc*). × 198. Fig. 60. L.s. upper part of the ovule; the large cells below the endosperm proper (micropylar chamber) are portions of secondary haustoria (*sh*). × 198. Fig. 61. Endosperm with 2-celled proembryo; chalazal chamber has also become cellular. × 198. Figs. 62-64. Later stages in the development of endosperm. × 46. Fig. 65. Whole mount of placental column showing the position of endosperm. × 20.



FIGS. 66-73 — Embryogeny. (Except for Figs. 68 and 72 which are from microtome sections, rest are from whole mounts.) Fig. 66. Zygote, the orientation of the spindle will lead to a vertical division. × 478. Fig. 67. Two-celled proembryo. × 478. Fig. 68-72. Stages showing progressive development of the proembryo. × 478. Fig. 73. Mature embryo. × 26.

Usually endosperm formation is initiated in all the ovules in an ovary but ultimately it reaches maturity in only one of them.

EMBRYO — The zygote divides only after the endosperm has become quite massive. The first division is longitudinal (Figs. 66, 67), and both the daughter cells divide transversely (Fig. 68) forming a quadrant (Fig. 69). This is followed by the octant stage (Fig. 70). Further divisions (Fig. 71) give rise to a globular proembryo (Fig. 72). The mature embryo is dicotyledonous (Fig. 73). It is interesting to note that there is no trace of a suspensor at any stage.

PERICARP — At the mature embryo sac stage the ovary wall consists of 17-20 layers of parenchymatous cells (Figs. 74, 75). Stomata are present on the inner as well as the outer epidermis. The outer hypodermal layer contains tannin. Two to three layers, next to the inner epidermis, undergo periclinal divisions resulting in an appreciable increase in the thickness of the pericarp. At the globular stage of the proembryo it is distinguishable into three zones — epicarp, mesocarp and endocarp (Figs. 76, 77). The epicarp comprises six to seven layers of parenchymatous cells which enlarge considerably and become loosened at maturity. The vascular strands pass through this region. At first the mesocarp consists of parenchymatous cells but during maturation of the fruit they become transformed into stone cells. The latter have a narrow lumen and show numerous pit canals (Fig. 79). The broad parenchymatous endocarp is consumed by the endosperm so that only the degenerated remains of this layer can be seen in a mature fruit (Figs. 78, 79).

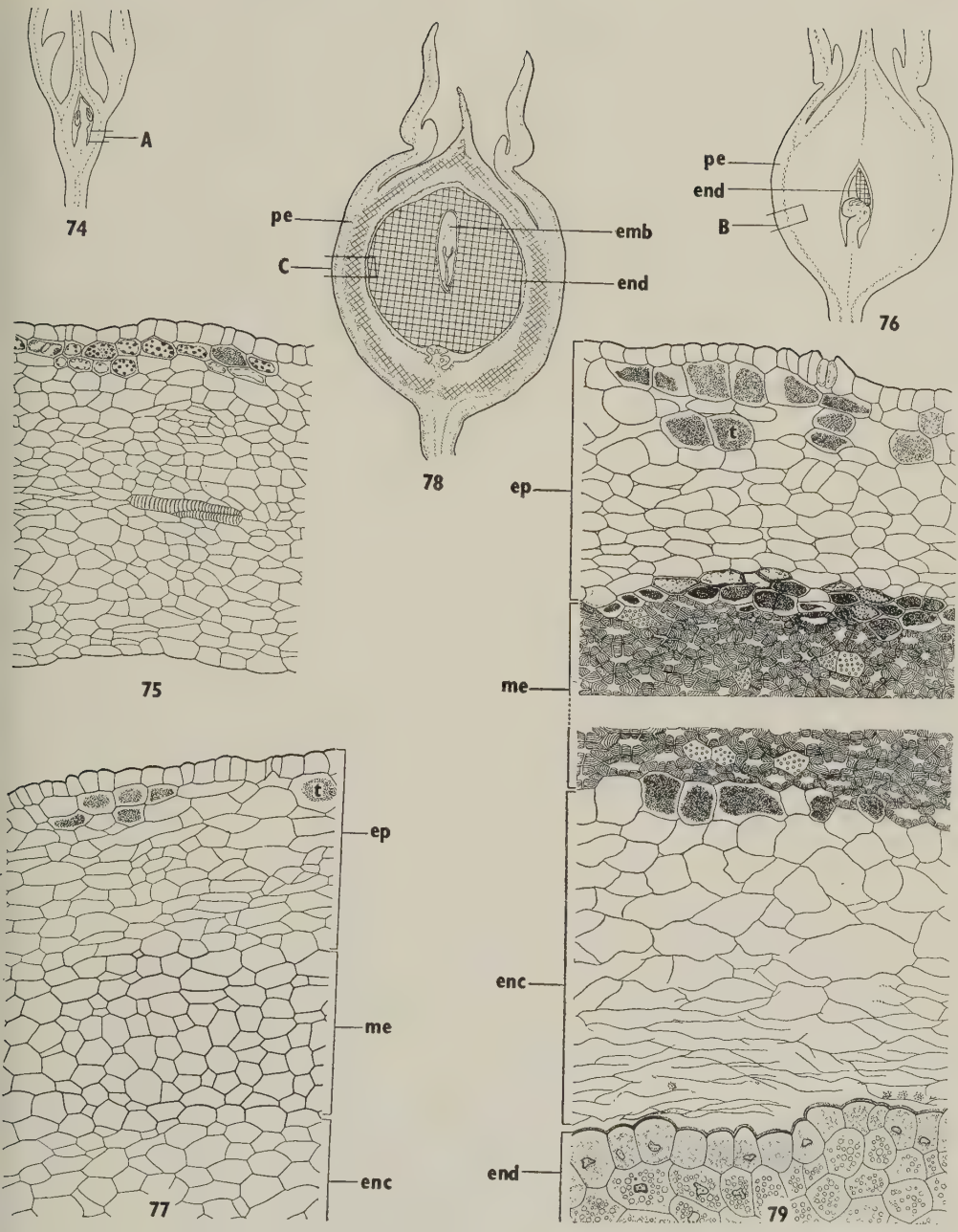
Discussion

In most santalaceous plants there is a multicelluar archesporium but only a few cells function as megaspore mother cells. In *Comandra* only one or two archesporial cells are formed.

The development of the embryo sac corresponds to the Polygonum type. The formation of a chalazal caecum is a very characteristic feature of the Santalaceae. It develops from the chalazal end at a level just above the antipodal cells. However, in *Comandra umbellata* the haustorium arises laterally from the upper part of the embryo sac just below the egg apparatus and invades the placental column. Another feature peculiar to this species is that the micropylar end of the embryo sac remains confined to the ovule, differing from the condition in *Santalum*, *Osyris* and *Thesium*.

In most of the plants the division of the primary endosperm nucleus is followed by a transverse wall which divides the embryo sac into a micropylar and a chalazal chamber. The latter acts as a haustorium and the micropylar chamber forms the bulk of the endosperm (*see* Guignard, 1885; Schulle, 1933; Rutishauser, 1937; Rao, 1942; Paliwal, 1956).

In *Comandra* the first wall, after the division of the primary endosperm nucleus, is laid down at the junction of the embryo sac proper and the lateral caecum. The partition of the embryo sac into a larger



FIGS. 74-79 — Pericarp. (*emb*, embryo; *enc*, endocarp; *end*, endosperm; *ep*, epicarp; *me*, mesocarp; *pe*, pericarp; *t*, tannin.) Fig. 74. L.s. ovary at mature embryo sac stage. × 8. Fig. 75. Magnified view of region marked *A* in Fig. 74. × 140. Figs. 76, 78. L.s. fruits at early globular and dicotyledonous stages of embryo. × 8. Fig. 77. Enlargement of portion marked *B* in Fig. 76; note the delimitation of epicarp, mesocarp and endocarp. × 140. Fig. 79. Magnified portion of epicarp and endosperm from region marked *C* in Fig. 78. × 140.

chalazal and a smaller micropylar chamber occurs only after the second division. The micropylar chamber gives rise to the endosperm proper while the chalazal normally remains undivided. The development of secondary endosperm haustoria, from the derivatives of the primary haustorium, is also noteworthy. According to Paliwal (1956) in *Thesium* secondary haustoria arise from the cells of the endosperm proper which lie in the vicinity of the embryo. These become enlarged, vacuolated and multinucleate and later the nuclei become hypertrophied.

Although in *Comandra*, like *Santalum*, endosperm formation is initiated in all the embryo sacs of an ovary, it reaches maturity in only one of them. Paliwal (1956) points out that in *Santalum* the development of the endosperm may continue in all the sacs in an ovary and ultimately all of them may fuse giving rise to a composite mass, as seen in the Loranthoideae (Maheshwari, Johri & Dixit, 1957). So far this is the only record of its type in the Santalaceae.

Unlike other members of the Santalaceae, in *Comandra* the first division of the zygote is longitudinal recalling the condition in the Loranthaceae and Balanophoraceae (*see* Maheshwari, 1950). However, unlike the Loranthaceae the first division is not followed by repeated transverse divisions, the proembryo does not undergo downward elongation, and a suspensor is absent. The 2-celled proembryo passes through the quadrant, octant, globular and heart-shaped stages, finally producing a typical dicotyledonous embryo.

The structure of the gynaecium led Engler & Prantl (1889) and Engler & Diels (1936) to place the genus *Comandra* in the tribe Osyrideae along with *Osyris* and *Santalum*. On the other hand, on the basis of the nature of the placental column and the ovule, Van Tieghem (1896) erected four tribes: Santalées, Osyridées, Thesiées and Comandrées.

In *Comandra*, features such as (i) the long, twisted placental column bearing two to four anatropous ovules, (ii) presence of a lateral caecum in the embryo sac, (iii) occurrence of secondary endosperm haustoria, and (iv) longitudinal division of the zygote, clearly indicate that this genus has very little in common with other members of the Osyrideae. Members of the latter are characterized by (i) a short placental column bearing three ovules; (ii) presence of a chalazal caecum; (iii) absence of secondary haustoria, and (iv) transverse division of the zygote. Therefore, the assignment of *Comandra* to a separate tribe Comandreae, as had been done earlier by Van Tieghem (1896), is quite justifiable on embryological grounds.

Summary

Comandra umbellata has cymose inflorescences and the flowers are typically pentamerous but tetra- or hexamerous conditions may also occur. The stamens are opposite and isomeric with the perianth lobes.

The ovary is semi-inferior and unilocular with two to four ovules borne on a free central twisted placental column.

The anther wall comprises four to six layers — the epidermis, endothecium with feebly developed fibrous thickenings, one to three middle layers and the glandular tapetum. At maturity the adjacent pollen sacs become confluent and dehiscence occurs by two longitudinal slits.

The microspore mother cells undergo simultaneous reduction divisions producing decussate or tetrahedral tetrads. Cytokinesis occurs by furrowing. The pollen grains are rounded, have three germ pores and are shed at the 2-celled stage.

The ovule shows a clearly demarcated integument. There are one or two archesporial cells but only one of them functions. The development of the embryo sac conforms to the Polygonum type and the antipodal cells are ephemeral. A lateral caecum originates from the embryo sac (on the funicular side) slightly below the level of the egg apparatus. It grows through the ovule and enters the placental column.

The endosperm is Cellular and the first division is followed by a wall formed at the junction of the lateral caecum and the embryo sac proper. A small cell is then

cut off in the caecum. Its derivatives give rise to secondary endosperm haustoria while the rest of the caecum functions as the primary haustorium. Subsequently, the embryo sac gets divided into micropylar and chalazal chambers but only the former contributes to the endosperm proper.

The first division of the zygote is longitudinal; the embryo is dicotyledonous and lacks a suspensor.

Due to the aggressive activity of the endosperm, the ovular tissue, placental column and the parenchymatous endocarp are used up and the seed becomes ' naked '. In a ripe fruit the pericarp consists of the parenchymatous epicarp and the stony mesocarp.

On embryological grounds it seems proper to segregate *Comandra* from the tribe Osyrideae and place it in a new tribe Comandreae.

I am indebted to Professor P. Maheshwari and Dr B. M. Johri for putting the material of *Comandra* at my disposal and for valuable suggestions and comments throughout the course of this investigation.

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THE EMBRYOLOGY OF *HYPERICUM PATULUM* THUNB. AND *H. MYSORENSE* HEYNE

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Previous work on the embryology of eight species of *Hypericum* relates mainly to the development of the endosperm and the origin and behaviour of the 'basal cyst'. Schnarf (1914) described the embryo sac, endosperm and seed coat of *H. perforatum* and *H. maculatum*. Palm (1922) considered the endosperm of *H. japonicum* to be Helobial while Dahlgren (1923) observed Nuclear type in *H. kalmianum*. Stenar (1938) studied *H. acutum* and corrected some of the observations of Palm. Swamy (1946) confirmed these observations in *H. mysorense*. Recently, Govindappa (1956) reinvestigated *H. japonicum* and traced various aspects of the life history. The embryo development of *H. perforatum* and *Androsaemum officinale* has been worked out by Souèges (1925, 1936), and ovule and seed coat of *Androsaemum officinale* by Crété (1936). The present work deals with the comparative account of the embryology of *H. patulum* Thunb. and *H. mysorense* Heyne.

Materials and Methods

The material of *H. patulum* was collected from the Government Botanic Gardens, Ootacamund, Nilgiris; *H. mysorense* at Nandi Hills and Kemmannagundi, Mysore State. Both of them were fixed in formalin-acetic-alcohol and Bouin's fluid. Sections were cut at a thickness of 10-16 microns and stained in iron-haematoxylin, followed by a counterstain of erythrosin in clove oil. Considerable difficulty was experienced in sectioning fruits due to the presence of tannin in the ovary wall, placentae and seed coat.

Observations

In both the species the floral parts arise in acropetal succession. The petals show stalked multicelluar glands with richly cytoplasmic cells containing prominent nuclei and dark staining bodies (Fig. 1).

Microsporogenesis and Male Gametophyte — The stamens are indefinite in number and arranged in three fascicles. Each stamen bears a globular, dithecous anther on a long filament. A transection of the young anther lobe shows a plate of four hypodermal archesporial cells (Fig. 2) which divide to form the primary parietal and primary sporogenous layers. The former by further periclinal divisions gives rise to endothecium — which later on becomes fibrous —, two ephemeral middle layers and the glandular binucleate tapetum (Figs. 3, 4, 27, 28). The epidermal cells contain dark staining bodies, and the middle layers disorganize early (Fig. 28).

The sporogenous cells divide once or twice to produce the microspore mother cells which undergo the usual reduction divisions (Figs. 5, 29). Quadripartition takes place by centripetal cleavage furrows and the tetrads show a tetrahedral (Fig. 6), isobilateral (Figs. 7, 30), or sometimes decussate arrangement (Figs. 8, 31).

A young microspore has dense cytoplasm and a centrally situated nucleus. It divides adjacent to the wall, resulting in the organization of a small lenticular generative cell and a large tube cell. The pollen is shed at the 2-celled stage and is tricolporate with a thick exine and a thin intine (Figs. 9, 32).

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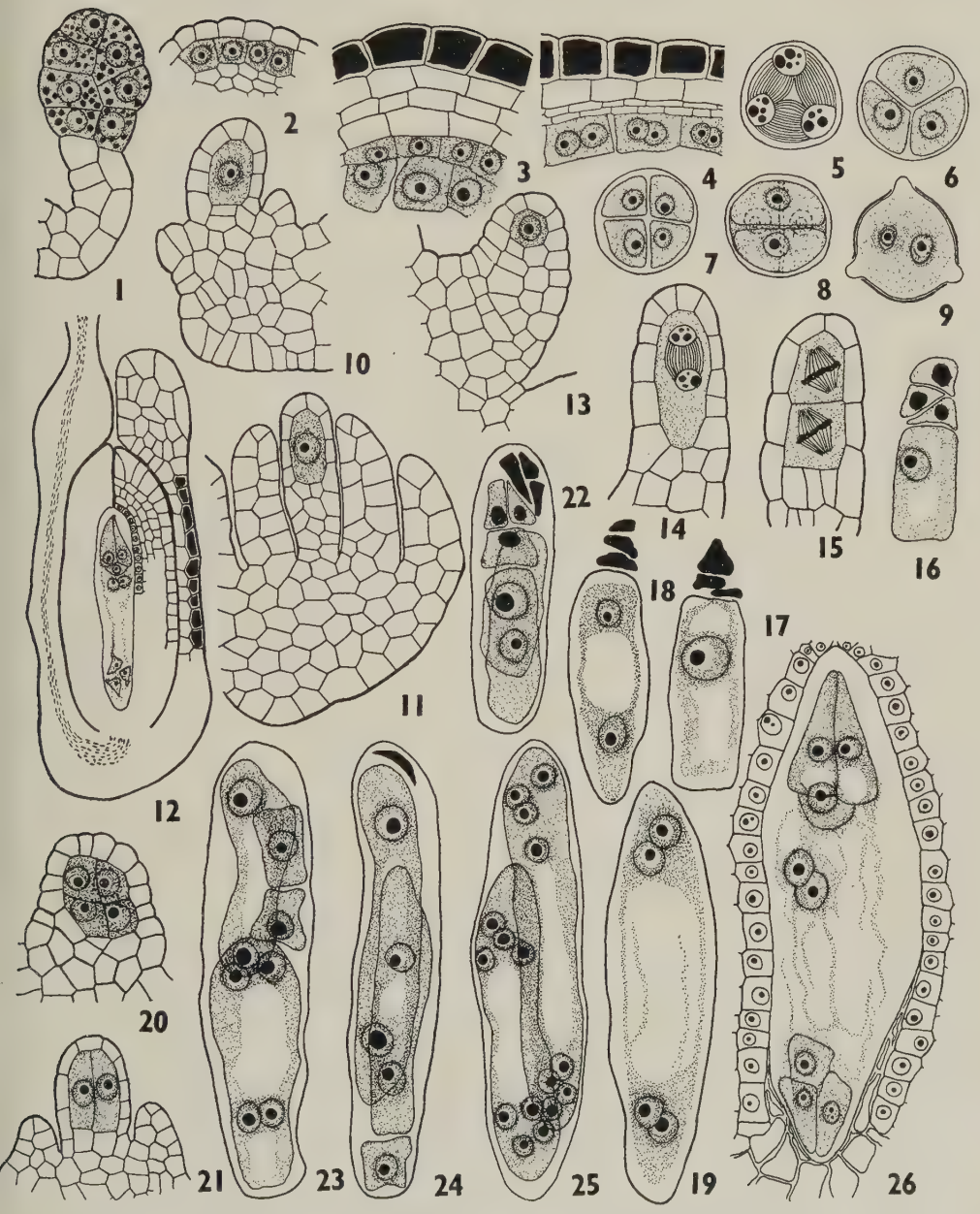
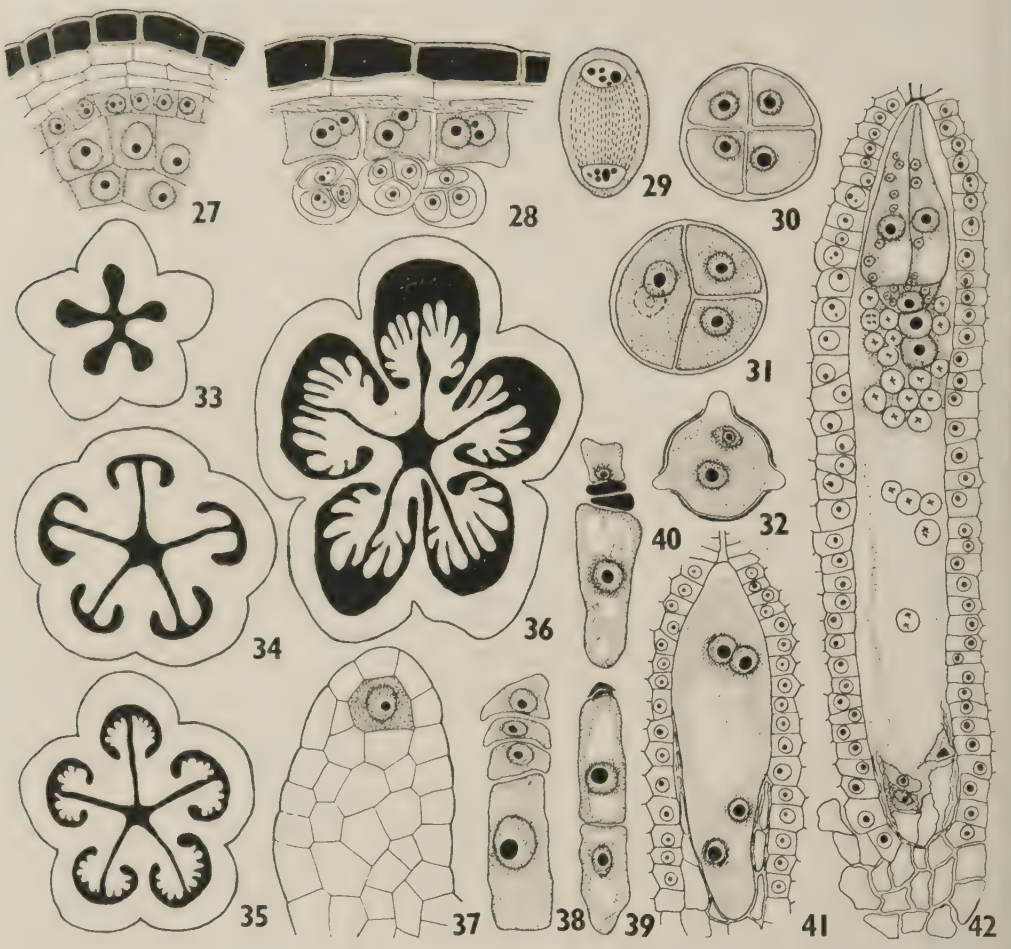


FIGS. 1-26 — *Hypericum patulum*. Fig. 1. L.s. gland on petal. × 500. Fig. 2. T.s. part of anther lobe showing archesporium. × 500. Figs. 3, 4. Same, later stages. × 500. Fig. 5. Meiosis II. × 1500. Figs. 6-8. Tetrahedral, isobilateral and decussate tetrads. × 1500. Fig. 9. Mature pollen grain. × 1500. Figs. 10-12. L.s. ovules. × 500, × 500, × 350. Figs. 13. L.s. young nucellus showing archesporial cell. × 500. Fig. 14. Megaspore mother cell. × 500. Fig. 15. Dyads in division. × 500. Fig. 16. Linear tetrad. × 1000. Figs. 17-19. Functioning megaspore, two and four-nucleate embryo sacs. × 500. Figs. 20-21. Four archesporial cells and two megaspore mother cells. × 500. Fig. 22. Twin tetrads, one is ' T ' shaped. × 500. Figs. 23-25. Twin embryo sacs. × 500. Fig. 26. Mature gametophyte. × 500.



FIGS. 27-42 — *Hypericum mysorense*. Fig. 27. T.s. young anther wall. × 500. Fig. 28. Same at a later stage. × 500. Fig. 29. Dyad. × 1500. Figs. 30-31. Tetrahedral and isobilateral tetrads. × 1500. Fig. 32. Mature pollen grain. × 1500. Figs. 33-36. T.s. ovary showing the growth of placentae and ovules. × 50, × 50, × 35, × 15. Fig. 37. Megaspore mother cell. × 500. Figs. 38-40. Linear tetrads. × 500. Fig. 41. Four-nucleate embryo sac. × 500. Fig. 42. Mature embryo sac. × 500.

OVARY — In *H. patulum* the gynoecium is tricarpellary while in *H. mysorense* it is pentacarpellary. In both the species the ovary is unilocular and the ovules are arranged on parietal placentae. In the ovary the placental tissues grow into the locule (Fig. 33), and the ingrowths expand and recurve downward towards the ovary wall in the form of expanded arrowheads (Fig. 34). The cells of the epidermal layer of the placentae have conspicuous nuclei and dense cytoplasm. The ovular primordia are restricted to those regions which face the ovary wall; and the apical portions of the placentae in the central region of the locule are devoid of ovules (Figs. 35, 36). The ovules are anatropous, tenuinucellate, and compactly arranged. The ovule originates as a conical outgrowth and as the megaspore mother cell becomes prominent, the initials of the inner and outer integu-

ments make their appearance (Fig. 10). The outer integument surpasses the inner but finally both contribute to the formation of the micropyle (Fig. 12).

To start with, both integuments are two-layered (Fig. 11) but at the mature embryo sac stage the inner becomes 5-6-layered (Fig. 12). The developing meagspore gradually enlarges destroying the surrounding nucellar cells. At about the 4-nucleate stage of the gametophyte, the inner epidermis of the inner integument forms the endothelium (Fig. 41) which becomes very prominent when the embryo sac has fully organized (Figs. 26, 42).

MEGASPOROGENESIS AND FEMALE GAMETOPHYTE — The hypodermal archesporial cell (Fig. 13) functions directly as the megaspore mother cell (Figs. 14, 37). It divides (Figs. 14, 15) to form a linear tetrad of megaspores of which the chalazal functions (Figs. 16, 38). In *H. mysorense* some examples were noticed where the uppermost or the third and fourth megaspores showed signs of further

FIGS. 43-62 — *Hypericum patulum*. Fig. 43. Embryo sac showing double fertilization. × 233. Figs. 44-49. Development of free nuclear endosperm. × 233. Figs. 50-53. Formation of the cyst. × 1000, × 1000, × 500, × 500. Fig. 54. An unusually elongated cyst. × 500. Fig. 55. L.s. ovule showing merging of cyst with the rest of the endosperm. × 75. Figs. 56-59. Gradual spreading of the cyst. × 333. Figs. 60-61. Micropylar and chalazal region enlarged to show cellular endosperm. × 333, × 366. Fig. 62. L.s. mature seed. × 133.

development (Figs. 39, 40). The functioning megaspore undergoes three more divisions (Figs. 17-19, 38-42) resulting in an embryo sac of the Polygonum type (Maheshwari, 1950; Schnarf, 1914; Govindappa, 1956).

Sometimes both the species show a group of archesporial cells (Fig. 20) and the development of more than one cell leads to the formation of multiple gametophytes (Figs. 21-25). However, usually only one embryo sac attains maturity.

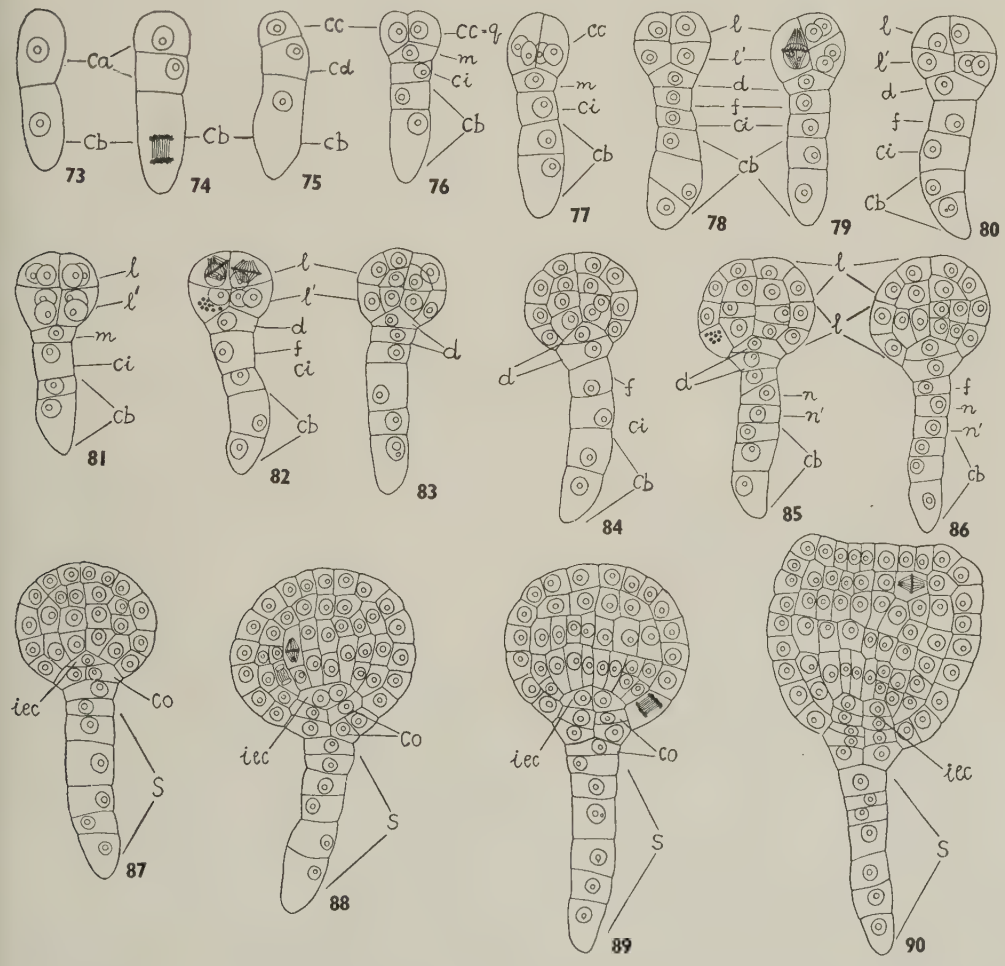
As compared with *H. patulum*, the embryo sac of *H. mysorense* is much longer, contains abundant starch grains, the synergids are elongated, and the polar nuclei fuse adjacent to the egg apparatus (Figs. 26, 42).

Fertilization is porogamous. The pollen tube destroys one of the synergids while

Figs. 63-72 — *Hypericum mysorense*. Fig. 63. Embryo sac showing the primary endosperm nucleus in metaphase; note the starch grains. × 350. Figs. 64-67. Two-, 4- and 8-nucleate cysts. × 500. Fig. 68. Completely developed cyst. × 500. Fig. 69. Gradual loosening and spreading of the cyst. × 500. Fig. 70A. L.s. ovule to show the merging of the cyst. × 10. Fig. 70B. Magnified view of the cyst. × 350. Fig. 71. Division of endosperm nuclei after merging of the cyst. × 350. Fig. 72. Chalazal region enlarged to show cell formation extending even to the region of the cyst. × 350.

the other persists for a considerable time and disorganizes only after about 16 endosperm nuclei have been formed (Figs. 48, 49). Double fertilization has been observed (Fig. 43).

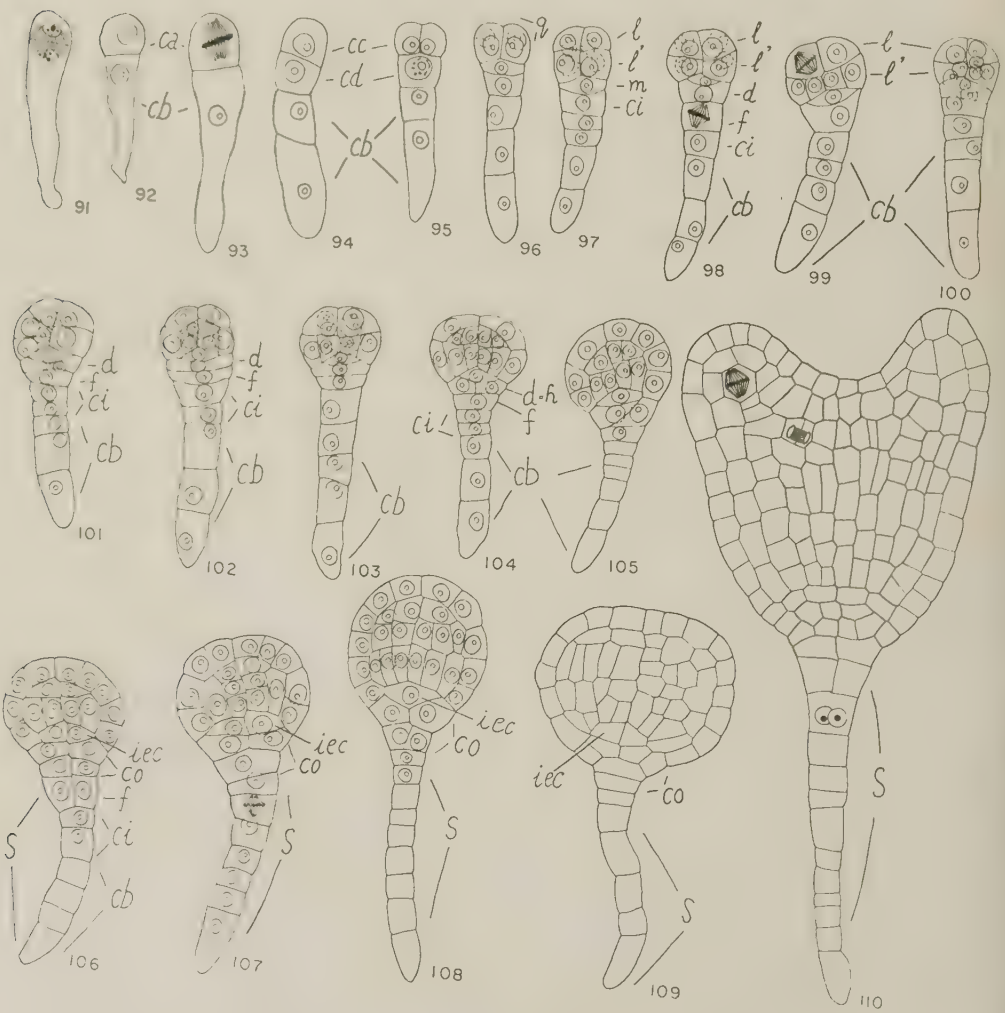
ENDOSPERM — The primary endosperm nucleus is situated in the centre of the embryo sac adjacent to the zygote. The first division is not followed by wall formation and the two daughter nuclei move apart (Fig. 44) and undergo two more divisions to form eight nuclei (Figs. 44-46). One of these, located at the chalazal end, gathers dense cytoplasm forming a cyst (Figs. 47, 50-53). Thus, in *H. patulum* cyst formation starts at the 8 or 16-nucleate stage (Figs. 47, 48), the former condition being more common. Later the cyst assumes a distinct top-shaped appearance and contains 16-32 free endosperm nuclei (Fig. 52). Sometimes it becomes considerably elongated (Fig. 54). The cyst corresponds to the ' basal apparat ' described by Schnarf (1914) and other workers in different species of *Hypericum*.



FIGS. 73-90 — *Hypericum patulum.* (*co,* root cap; *iec,* initials of central cylinder of the root; *s,* suspensor.) Fig. 73. Two-celled proembryo. × 333. Figs. 74-90. Stages in development of embryo. × 333.

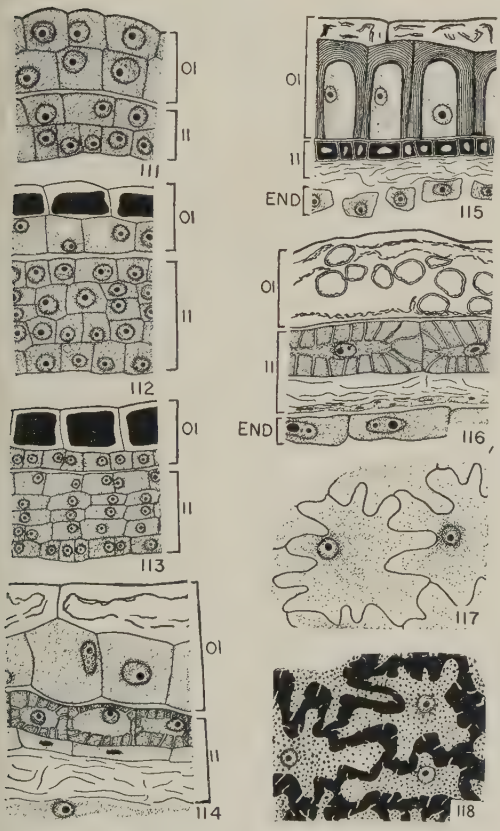
Meanwhile, the endosperm nuclei in the upper part of the embryo sac also divide and become distributed peripherally (Fig. 55). The broader upper portion of the cyst now begins to expand (Fig. 56) and large multinucleolate nuclei are produced by fusion (Figs. 57-59). Finally, the cyst merges with the general endosperm (Fig. 55). Wall formation is initiated at the micropylar end and continues towards the chalaza until the entire embryo sac becomes filled with cellular endosperm (Figs. 60-62). During the maturation of the seed, the endosperm is gradually used up by the embryo.

The development in *H. mysorense* is almost similar. Here the basal cyst is cut off when 16 endosperm nuclei have been produced and starch grains accumulate around them. By the time 32 nuclei are formed, the starch grains become



FIGS. 91-110 — *Hypericum mysorense*. (*co*, root cap; *iec*, initials of central cylinder of the root; *s*, suspensor.) Fig. 91. First division of zygote. × 333. Figs. 92-110. Stages in embryo development. × 333.

digested and are no longer recognizable. The endosperm cells adjacent to the cyst are larger and have elongated multinucleolate nuclei (Fig. 72). Both in *H. patulum* and *H. mysorense* the basal cyst gradually merges with the general endosperm and does not degenerate. Govindappa (1956) has made similar observations in *H. japonicum*. Swamy's (1946) report that in *H. mysorense* the conspicuous coenocytic cyst "persists for a long time, finally degenerating as such without merging into the general endosperm" is, therefore, not borne out by the present study.



FIGS. 111-118 — Figs. 111-115 of *H. patulum* and Figs. 116-118 of *H. mysorense*. (*ii*, inner integument, *oi*, outer integument; *end*, endosperm.) Figs. 111-115. Portions of integuments (l.s.) showing progressive development of seed coat. × 500. Fig. 116. Seed coat of *H. mysorense*. × 500. Figs. 117, 118. Sclerified cells from outer epidermis of inner integument. × 333.

EMBRYO — The zygote is densely cytoplasmic, and in *H. mysorense* it contains abundant starch grains. In both the species it elongates and undergoes a transverse division producing two superposed cells *ca* and *cb* (Figs. 73, 91, 92). Of these, *ca* divides first forming *cc* and *cd* (Figs. 75, 94). Shortly afterwards, *cb* also undergoes a transverse division (Figs. 76, 94), resulting in a quadrant which belongs to the C2 category of Souèges's system of classification. Now *cc* undergoes vertical divisions to form the quadrant *q* (Figs. 77, 96). Sometimes the first vertical division in *cc* is followed by a transverse wall (Fig. 78). By another division the quadrant gives rise to the octant (Figs. 79-81, 98). The upper tier *l*, comprising the superior octant, contributes to the cotyledonary portion *pco*; while the lower tier *l'*, comprising the inferior octant, contributes to the hypocotyledonary region *phy* (Figs. 83-86, 102-105).

By this time *cd* has divided transversely forming the tiers *m* and *ci* (Figs. 76, 97). On division *m* produces *d* and *f* (Figs. 78, 98). The derivatives of *d* give rise to the hypophyseal region of the embryo (Figs. 83, 84, 103, 104). Figs. 87-90 and 105-110 represent stages leading to the differentiation of different parts of the embryo, namely *icc*, *iec* and *co*. The suspensor is derived from the basal cell *cb* and partly from *ci* which is a derivative of the apical cell. In *H. mysorense* it consists of 12-14 cells whereas in *H. patulum* it shows only 5-6 cells.

The development of the embryo may be assigned to Megarche type IV in group 9 of the second period of Souèges's system and is in conformity with the course of development described for the other members of this family (Souèges, 1925a, 1936; Govindappa, 1956). The mature embryo is elongated, straight, and dicotyledonous and occupies almost the entire seed cavity (Fig. 62).

SEED — Both the integuments contribute to the formation of the seed coat. After fertilization, the epidermal cells of the outer integument elongate radially (Figs. 114-116) and the dark staining

FIGS. 119-126 — *H. patulum*. Figs. 119-122. Diagrams of t.s. of ovary wall in the region of the groove. × 50, × 20, × 20, × 10. Figs. 123-126. Portion of same enlarged to show cellular details. × 250.

bodies gradually disappear. In *H. mysorense* (Fig. 116) the inner layer of the outer integument disintegrates; in *H. patulum*, on the other hand, its cells enlarge (Fig. 114). The outer epidermis of the inner integument becomes thick-walled while the remaining layers disintegrate (Figs. 115, 116). The testa is composed of two layers in *H. patulum* and only one in *H. mysorense*. In the former the inner layer develops thickenings on the outer and inner tangential walls. In both species the tegmen is single-layered with lignified thickenings and simple pits (Figs. 115, 116).

FRUIT — At an early stage the ovary wall shows three grooves opposite to the placentae in *H. patulum* and five in *H. mysorense*. In the beginning, the cells in this region of the groove are of uniform size (Fig. 123). As the groove becomes more pronounced, the cells situated immediately below it divide forming a plate of small cells which later on elongate (Fig. 124). In a fruit this plate of cells gradually shrinks and disorganizes (Fig. 125) leading to the dehiscence of the capsule (Figs. 125, 126). The innermost layer of the pericarp develops fibrous thickenings.

Summary

In *Hypericum patulum* and *H. mysorense* the anther tapetum is glandular and binucleate. Quadripartition of the microspore mother cells occurs by centripetal cleavage furrows. Isobilateral, tetrahedral, and decussate tetrads are formed. The pollen grain is tricolporate and is shed at the two-celled stage.

The parietal placentae bear a number of bitegmic, anatropous, tenui-nucellate ovules. The hypodermal archesporial cell functions directly as the megaspore mother cell and the development of embryo sac follows the Polygonum type.

The first division of the primary endosperm nucleus is not followed by wall formation. The endosperm is Nuclear and not Helobial as previously reported by Palm (1922). A chalazal cyst is formed as in the other species of *Hypericum*. It is initiated at the 8-nucleate stage of the endosperm in *H. patulum* and at the 16-nucleate stage in *H. mysorense*. The cyst does not degenerate but gradually merges with the general endosperm. Wall formation occurs when the cotyledons are about to be differentiated. The endosperm is used up by the growing embryo.

The development of the embryo is described in detail. The hypophyseal region is formed by the cell *d*. The derivatives of *cb*, *ci*, and *f* form a suspensor of five to six cells in *H. patulum* and 12-14 cells in *H. mysorense*.

The seed coat consists of three layers in *H. patulum* and two layers in *H. mysorense*.

It gives me great pleasure to thank Professor P. Maheshwari for valuable suggestions and for critically going through the slides and manuscript, Professor K. N. Narayan for kind encouragement and interest, and Doctors M. Anantaswamy Rau and K. Subramanyam for helpful suggestions. Thanks are also due to Mr B. G. Narayana Menon, Curator, Government Botanic Gardens, Ootacamund, for permitting me to collect the material and to the Systematic Botanist, Agricultural College, Coimbatore, for identification of the plants used in this study. To the authorities of the Mysore University I am grateful for the award of a research fellowship.

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STUDIES OF MORPHOGENESIS IN THE NYMPHAEACEAE. I—INTRODUCTION: SOME ASPECTS OF THE MORPHOLOGY OF *NUPHAR LUTEA* (L.) SM. AND *NYMPHAEA ALBA* L.

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The present paper is intended as an introduction to the morphogenetic phenomena disclosed by a preliminary re-examination of the commonest British members of the Nymphaeaceae, *Nuphar lutea* (L.) Sm. and *Nymphaea alba* L. These species belong to the tribe Nymphaeoideae of this somewhat heterogeneous family [i.e. to the Nymphaeaceae *sensu stricto* of Li (1955)].

The outstanding morphological interest of the Nymphaeaceae has long been recognized, and much published work exists which deals with the taxonomy and various aspects of the anatomy and morphology of the family, including embryology, germination and seedling development, and heteroblastic leaf development. This literature will not be fully reviewed here, but will be cited in the appropriate passages of the text. Conard (1905) has reviewed the history of waterlilies and also the relevant earlier literature. The Nymphaeaceae are also of phylogenetic interest because of their taxonomic position as relatively primitive dicotyledons possessing features of embryogeny and anatomy which certain authors have considered to be more closely allied to the monocotyledonous condition (e.g. Cook, 1906; Henfrey, 1852; Schaffner, 1904; Trécul, 1845, 1852, 1854; York, 1904). The former economic importance of water-lilies (*see* Irvine & Trickett, 1953), their antiquity, their widespread distribution and their frequent cultivation as ornamental plants have all served to stimulate investigation.

Notwithstanding this earlier work, it seems likely that a re-examination of certain aspects of the morphology and anatomy of water-lilies, involving the application of newer methods and concepts, may be profitable. It may be noted that the shoot apices of *Nuphar* and *Nymphaea* are attractive materials for

morphogenetic studies because of their comparatively large size and their location at the tip of a solid rhizome. Furthermore, the apices do not easily become desiccated on exposure to the atmosphere or to a lamp, and they will withstand surgical treatments at least to some extent, as Wardlaw (1952) has already shown.

Materials and Methods

Rhizomes of various sizes of *Nuphar lutea* were collected from meres and canals in Cheshire and Lancashire, England. Similar material of *Nymphaea alba* was collected from Cheshire, Lancashire, East Lothian and Sutherland. Seedling plants of *Nuphar lutea* were obtained during late August from a mere in Cheshire; seedlings and young plants of *Nymphaea alba* were collected in early August from a loch in Sutherland, Scotland.

For examination of the mature rhizomes the roots were excised and the rhizomes carefully scrubbed. The hairs which persist on the interfoliar regions of the rhizome of *Nymphaea alba* were removed as far as possible. To expose the rhizome apices, all the young leaves and flowers except a small number surrounding the apex were excised with a sharp scalpel, and the numerous mucilaginous hairs present in both species were removed with fine forceps or flattened with a sharpened sewing needle. Camera lucida tracings were made under a binocular microscope at a magnification of $\times$ 65.

Throughout these papers the terminology of Snow & Snow (1931) will be followed: that is, young visible leaf (and flower) primordia will be called P_1, P_2, P_3 etc., P_1 being the youngest; prospective primordium sites will be called I_1, I_2, I_3, etc., I_1 being the first to arise.

Rhizome Morphology

In order to provide a coherent introduction and some basic illustrations for these studies, it seems desirable briefly to restate here certain aspects of rhizome morphology and to treat any newer observations in more detail, but reference should also be made to earlier accounts of the rhizomes of these plants, notably to the work of Raciborski (1894a) and Heslop-Harrison (1955a, 1955b).

The yellowish, horizontal rhizome of *Nuphar lutea*, which is somewhat dorsiventrally flattened, bears the scars of petioles and peduncles. The latter, which are circular in shape, can be seen to occur in leaf positions in the parastichies (Figs. 1, 3). In large rhizomes the phyllotactic system is (3+5). A small scale-like bract is present at the base of the mature peduncle. Stipules are absent in *Nuphar*. Adventitious roots are present below the leaf scars on the lower side of the rhizome; they are not usually associated with peduncle scars, and if present are much less numerous. Trécul (1845) states that root primordia are associated with all leaf primordia, but develop only below those on the lower side of the rhizome, unless the latter, which is normally epigeal, is deeply buried. Flowers are not restricted to the upper side of the rhizome, as some authors state, but also occur laterally and on the lower side. They usually occur in pairs separated on the genetic spiral by one leaf, preceded and succeeded by a greater number of leaves (5 to 22, usually about 8 to 11). This is said to constitute one season's growth (Grainger, 1947). Flowers may also occur

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FIGS. 1-2 — Fig. 1. Upper surface of part of a rhizome of *Nuphar lutea* from which the adventitious roots and the outer leaves and flowers have been removed. A small lateral rhizome, the product of an axillary bud, is present on the right. The circular scars of the peduncles can be distinguished from the elliptical petiole scars, and can be seen to occur in leaf positions in the parastichies. Note the variation in diameter of the rhizome. $\times \frac{1}{2}$. Fig. 2. Upper surface of part of a rhizome of *Nymphaea alba* from which the adventitious roots and persistent hairs have been removed. The circular peduncle scars, which have no associated root scars, occupy leaf sites in the parastichies. The petiole scars, which are slightly more elliptical, have root scars beneath them and an adaxial flange which is the scar of the membranous intrapetiolar stipule. $\times \frac{1}{2}$.

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FIGS. 1-2.

singly or in threes; occasionally they may constitute three successive organs in the genetic spiral, but more often they are separated, as in the former instance, by one intervening leaf. Raciborski (1894a) has already pointed out that characteristic arrangements of flowers and leaves occur in different species of waterlilies; further work on this aspect is in progress.

The width of the rhizome may fluctuate considerably; on the narrower parts the leaf and peduncle scars are set closer together (Figs. 1, 3). This may perhaps be a consequence of seasonal effects. In all instances fewer leaf and flower bases remain on the lower side of the rhizome, and these are set further apart, due to the greater growth of the intervening tissue (Fig. 3). This is a secondary effect, since the spacing of primordia at the shoot apex is symmetrical.

Occasional branching of the rhizome takes place by means of axillary buds (Figs. 1, 3), and is not dichotomous, as stated by Glück (1924). Buds are not formed in the axil of every leaf; in fact, they are usually of rather rare occurrence. It is noteworthy that these buds (each with its subtending leaf) occupy positions where flowers would be expected, and that they are always succeeded on the genetic spiral by the other flowers of the group; that is, where they occur they occupy the position of the first (oldest) flower of any group of two or three flowers, intervening leaves being present as before.

The mature rhizome of *Nymphaea alba* is dark brown to blackish in colour, covered with persistent hairs in the interfoliar regions; it is horizontal and of radial symmetry, though occasionally slightly flattened. The phyllotaxis of the large rhizomes examined was (5+8). The scars of both petioles and peduncles are approximately circular, but those of the peduncles have no root scars beneath them (Fig. 2). The flowers again occur in leaf positions (Figs. 2, 4), as many authors have previously recorded; in *Nymphaea* they are devoid of bracts. Scars of adventitious roots are present below petiole scars on all sides of the hypogeal rhizome, although they are somewhat larger on the lower surface. Adaxial to the petiole scars can be seen a small flange extending on either side of the leaf base (Fig. 2); this is the scar of the intrapetiolar membranous organ [the *stipula axillaris* of Irmisch (1853)] which is considered to represent fused stipules

FIG. 3 — Tracing of the scars of petioles and peduncles on part of a rhizome of *Nuphar lutea*, showing the much greater development of the interfoliar regions on the lower surface of the rhizome. The scars are more closely situated on narrower parts of the rhizome. *X* marks the centre of the upper surface. Leaf scars black; peduncle scars outlined; vegetative axillary bud stippled. Some petiole scars are indicated half on one side of the diagram and half on the other. × ⅓.

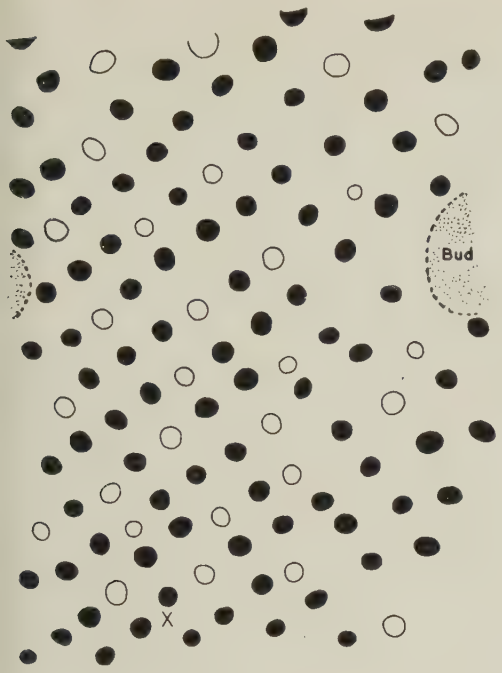

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FIG. 4 — Tracing of petiole and peduncle scars on part of a rhizome of *Nymphaea alba*, showing the occurrence of flowers in leaf positions and the comparatively symmetrical arrangement of the scars (compare Fig. 3). A vegetative bud occurring in a leaf site is also shown. *X* marks the centre of the upper surface of the rhizome. Petiole scars black; peduncle scars outlined; vegetative bud stippled. × ⅓.

(Arber, 1920; Gwynne-Vaughan, 1897). Thus in *Nymphaea alba* leaf scars may best be distinguished from those of flowers by their association with root and stipule scars.

Branching of the rhizome takes place by vegetative buds which are not axillary, as in *Nuphar*, but which occupy leaf positions. These vegetative buds are formed, as in *Nuphar*, in positions where flowers would be expected; they are usually infrequent, but on one rhizome examined ten such buds, interspersed with leaves and flowers, were present on the length examined, which comprised a total of 128 organs.

The terminal buds of large rhizomes of *Nuphar lutea*, collected in September and November, comprised some 30-40 unexpanded leaves and flowers which would develop in the following and successive seasons. In comparable material of *Nymphaea alba* examined in September, at least 50-60 unexpanded organs were found around the shoot apex.

Seedling Morphology

The development of germinating seedlings of these plants has been described in detail elsewhere (e.g. Arber, 1920; Conard, 1905; Gwynne-Vaughan, 1897; Heslop-Harrison, 1955a, 1955b; Trécul, 1845, 1852, 1854). An illustration of a later stage of development, in which the young plants of *Nymphaea alba* have formed small rhizomes, is presented here for comparison with the mature condition (compare Figs. 2, 5). Comparatively small numbers of unexpanded organs are present around the apex of young plants of both *Nuphar* and *Nymphaea*, e.g. of the order of 10 in large seedling plants which have formed small rhizomes.

Morphological Organization of the Shoot Apex

The shoot apices of both *Nuphar* and *Nymphaea* are comparatively large among Angiosperms (diameter $\pm$0·5 mm). Raciborski (1894a) gave a short description of the morphology of apices of both these genera, and Rauh & Rappert (1954) have recently dealt briefly with the apical anatomy of *Nymphaea*.

The exposed apex of *Nuphar lutea* is yellowish and flatly domed, surrounded by the spirally arranged primordia of leaves and flowers, which, as Raciborski (1894a, 1894b) and Schumann (1894) have already emphasized are not in contact with each other at their bases. To expose the apex and young primordia, it is necessary to remove large numbers of long, mucilaginous hairs; these are distributed generally round the older primordia in the sub-apical region, but are absent from the central meristematic regions from about the level of P_5 inwards. Young flower primordia are cylindrical in form, being distinguishable from leaf primordia at an early stage, usually even that of P_1,

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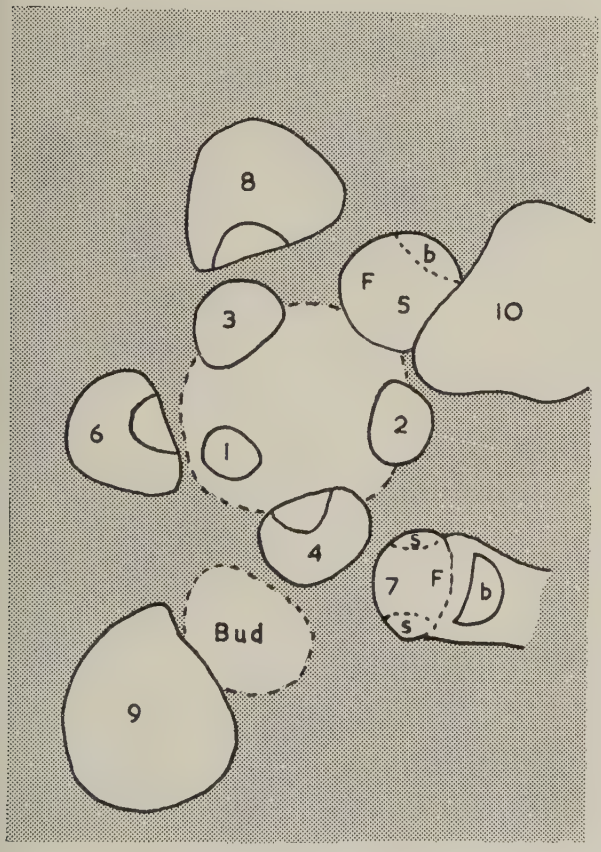
FIG. 5 Young plants of *Nymphaea alba* collected in early August from a loch in Sutherland. Small rhizomes have already been formed. Compare Fig. 2. × ½.

by their greater size and their radial symmetry. They are at first devoid of lateral organs and have no associated bract. Their vertical growth is initially rapid. Bract formation and organogenesis in the young flowers raise points of special interest and will be considered in a subsequent paper. In laying bare 168 apices of *N. lutea*, only 12 specimens were observed in which vegetative buds were present close to the apex, and six of these were from the same collection. Vegetative buds are extensive, flat growths, often situated somewhat laterally above the axil of the subtending leaf primordium (Fig. 6, primordium 9); they have not been observed in positions closer to the apex than the axil of P_7. There is apparently an initial period in which the bud meristem is formed, prior to the inception of leaf primordia; the latter subsequently originate in spiral sequence, in continuity with the axillant leaf. In Fig. 6, P_5 and P_7 are flower primordia, and a vegetative bud meristem is present in a position axillary and slightly lateral to P_9. In this apex the flower primordium at P_5 is giving rise to a primordium (*b*) in an abaxial position; in a subsequent paper it will be shown that this is the organ described in the literature as a bract. In the older flower at P_7 such an abaxial primordium (*b*) is also present, and two lateral sepal primordia (*s*) are being formed.

The shoot apex of *Nymphaea alba* is white in colour, more conspicuously domed than that of *Nuphar lutea*, and, together with the young primordia which surround it, situated in a somewhat sunken position due to the upgrowth of the more mature tissues around it. As in *Nuphar*, the young primordia are not in contact, as Church (1904) has already recorded. The distribution of the primordia and mucilaginous hairs is similar

to that in *Nuphar*. Membranous stipules can be observed in close association with the adaxial face of older leaf primordia, but are usually not externally visible in association with about ten of the youngest primordia. Young flowers are again distinguishable from leaf primordia by their size and shape. In 56 apices, only 3 were found in which vegetative buds occurred relatively close to the apex. These specimens were all from the same collection, and this fact, together with observations on *Nuphar*, suggests that the formation of vegetative buds is related to particular seasonal or environmental conditions. In describing the mature rhizome of *Nymphaea alba* it was stated that vegetative buds are not axillary, but occupy leaf sites. This observation, which does not seem to have been recorded previously, is fully borne out by direct observations of the shoot apex and by examination of



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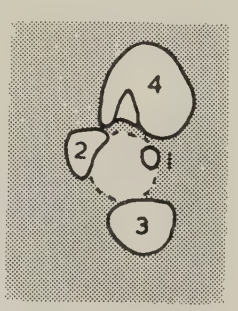

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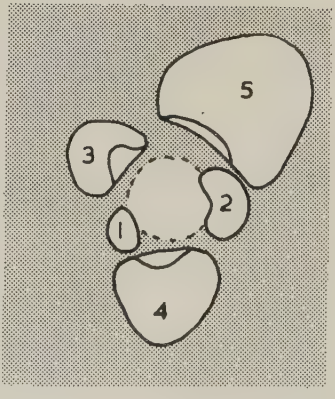

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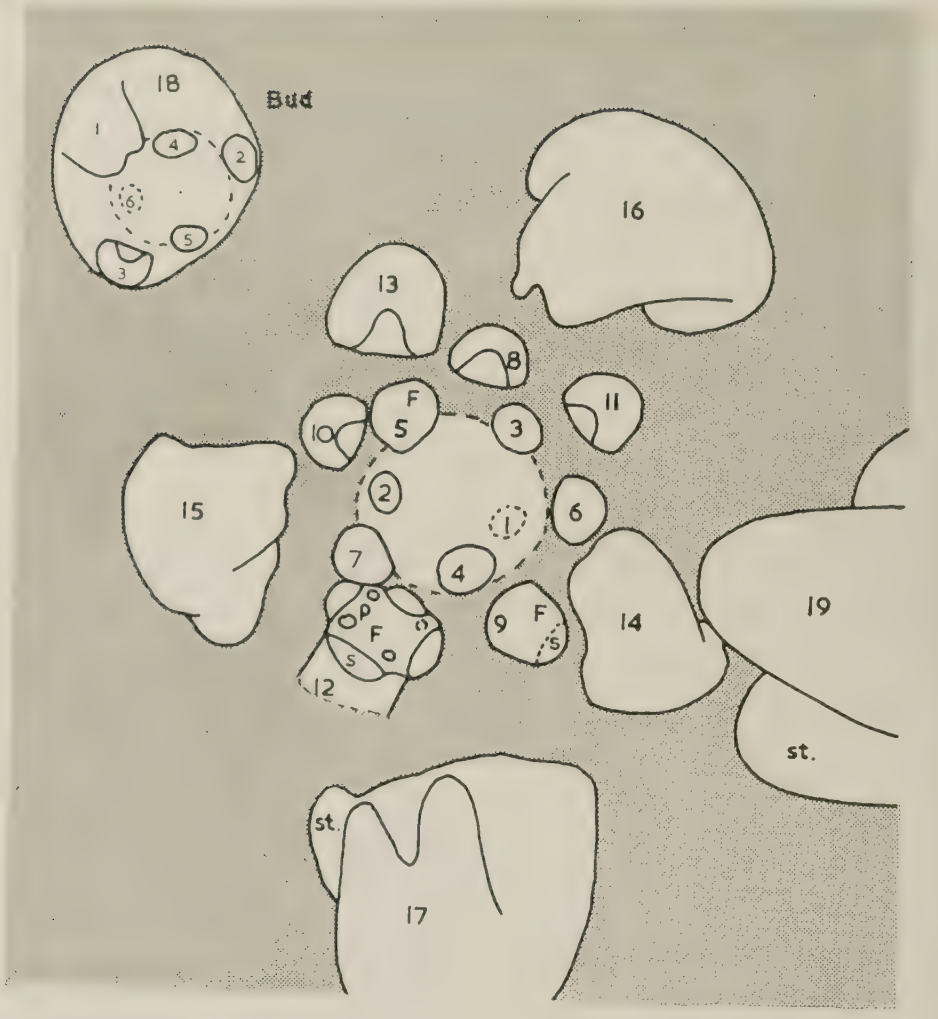
FIGS. 6-8 — *Nuphar lutea*. Fig. 6. Surface view of an apex of a large rhizome in which P_5 and P_7 are flower primordia (*F*), and a vegetative bud meristem is present axillary and slightly lateral to P_9. On the flower at P_5, the primordium of the bract (*b*) is present in an abaxial position; on that at P_7 the floral meristem has grown on, leaving the bract (*b*) situated abaxially on the peduncle. The primordia of the lateral sepals (*s*) are being formed. The extent of the apical meristem is indicated by a broken line; the sub-apical region is stippled; hairs are not shown. × 44. Figs. 7 and 8. Surface views of apices of seedling plants, showing the much smaller apical meristem, the larger size of the leaf primordia relative to the shoot apex, and the more marked increase in size and stage of development of successive leaf primordia. × 44.



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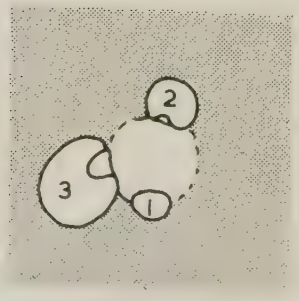

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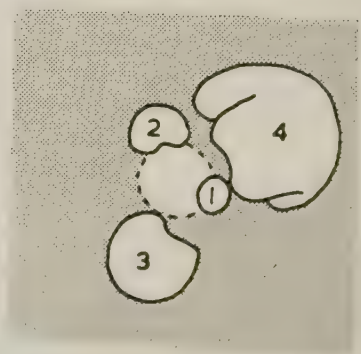

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FIGS. 9-12.

FIG. 13 — *Nymphaea alba*. L.s. specimen in which P_{12} was a vegetative bud. The section passes medianly through the shoot apex (centre), and the bud at P_{12} (right) with its first leaf primordium, which is in an abaxial position. P_8 is on the left. The bud meristem is slightly tilted towards the shoot apex. × 80.

transverse and longitudinal sections. In the specimens of *N. alba* examined, the vegetative buds occupied the positions of P_{12}, P_{13}, and P_{18} (Fig. 9) respectively. Vegetative buds of *N. alba* are more steeply domed in form than those of *Nuphar*, and, like leaf and flower primordia, are somewhat tilted towards the shoot apex, indicating greater abaxial growth (Fig. 13). Leaf formation on the bud takes place in spiral sequence, the first primordium occupying an abaxial position with respect to the main shoot apex (Figs. 9, 10).

Some preliminary observations of exotic species of *Nymphaea* suggest that vegetative bud formation is likely to prove of considerable morphological interest throughout the genus, but discussion of this matter will be deferred until a fuller study has been made.

Shoot apices of seedling plants of *Nuphar lutea* and *Nymphaea alba* are very much smaller in size than those of mature

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FIGS. 9-12 — *Nymphaea alba*. Fig. 9. Surface view of an apex of a large rhizome in which P_5, P_9 and P_{12} are flower meristems (F), and P_{18} is a vegetative bud with 6 leaf primordia (numbered in the order of their formation). The flower at P_5 is devoid of lateral organs, that at P_9 has its first sepal (s) in an abaxial position, and that at P_{12} has 4 sepals (s), and 4 petals (p) alternating with the sepals. The older leaf primordia have stipules (st); hairs are not shown. The approximate area of the apical meristem is indicated with a broken line; the sub-apical region is stippled. × 44. Fig. 10. Surface view of the vegetative bud at P_{18} in Fig. 9, with the main apex tilted so as to look directly down on to the apex of the bud, which is itself somewhat tilted towards the main shoot apex. Primordia are numbered in the order of their formation; the position of the parent shoot apex is towards the top of the page. × 44. Figs. 11 and 12. Apices of seedling plants, showing the small size of the apical meristem, the large size of leaf primordia relative to the shoot apex, and the rapid increase in size and complexity of successive primordia. × 44.

rhizomes, and leaf primordia are of large size relative to the shoot apex (compare Figs. 6-9, 11, 12). There is a greater size difference between successive primordia, and leaf development is apparently more rapid. This is more marked in seedling apices than in those of lateral buds (Figs. 10-12). Church (1904) states that seedling plants of *Nymphaea* have a (2+3) phyllotactic system, in contrast to the (5+8) system of the mature plant.

Discussion

The morphological interest of the Nymphaeaceae has been acknowledged for many years. Even a study restricted to the two common British species, *Nuphar lutea* and *Nymphaea alba*, has indicated that they are worthy of re-examination in the light of modern concepts, especially with regard to the organogenic relationships of leaves, flowers and vegetative buds. The occurrence of flowers in leaf positions in *Nymphaea* was noted as a phenomenon of unusual interest by Caspary (1891), Conard (1905), Eichler (1878), Raciborski (1894a), Troll (1937) and Velenovsky (1907). It has now been shown that, in *Nymphaea*, vegetative buds also originate at leaf sites, i.e. leaves, vegetative buds and flowers are homologous. The growth relationships involved are particularly interesting; for whereas the growth of leaves and flowers is ultimately determinate, that of vegetative buds is indeterminate. Moreover, since vegetative buds have been induced in leaf sites by surgical treatments of the fern *Dryopteris aristata* (Wardlaw, 1949, 1950; Cutter, 1956), it becomes important to discover whether similar experimental treatments can throw light on the factors controlling the normal development in these dicotyledonous species. The problem is likely to prove complex, since in *Nymphaea* vegetative buds, and in *Nuphar* vegetative buds together with their axillant leaves, are apparently formed at presumptive flower sites. *A priori* it seems that there is a special affinity between these organs which merits close analysis. The observations which have so far been made show clearly that, in addition to the experimental approach, which may include the aseptic culture of apical regions, studies of the external morphology and developmental anatomy of the same materials are likely to yield new findings of morphogenetic interest.

Ontogenetic studies of apical organization in ferns and in those angiosperms so far examined have already yielded important information. Even a brief examination of certain stages in the ontogeny of *Nuphar* and *Nymphaea* has indicated certain similarities with the ferns. In *Nuphar* and *Nymphaea* there is a considerable increase in the size of the shoot apex during ontogeny, as in many other plants (e.g. Abbe & Phinney, 1951; Allsopp, 1954; Wardlaw, 1948). The size of the leaf primordia relative to the shoot apex also changes, with an attendant change in phyllotaxis (Church, 1904; Richards, 1948), and the development of leaf primordia is more rapid in the seedling than in the mature plant. These observations also apply to some ferns (Crotty, 1955; Cutter, 1955; Steeves & Wetmore, 1953). The absence of contact between the young primordia at the shoot apex, already fully discussed by Raciborski (1894a, 1894b) and Schumann (1894), and also recorded by Church (1904), is also a condition more frequently found in ferns than in flowering plants (Snow, 1955). A further point is that during ontogeny the rate of inception of leaf primordia gradually becomes greater than the rate of seasonal utilization, so that a group of unexpanded organs surrounding the shoot apex is built up. It is already known that in the ferns *Dryopteris aristata* and *Osmunda cinnamomea* the unexpanded leaves present are sufficient for 3 or 4 years' expansion (Frazer, 1946; Steeves & Wetmore, 1953). In at least some of the specimens examined in the present work it is probable that in *Nuphar* the youngest primordia present would expand some 3 years later, and in *Nymphaea* perhaps 4 years later. The mechanism by which a group of slowly developing organs might be built up around the shoot apex has been discussed by Crotty (1955) for the fern *Acrostichum daneaefolium*, and it has also been shown that, under prolonged unfavourable conditions in which expanding leaf primordia are continuously removed,

apices of mature plants of *Dryopteris aristata* will revert to an organization similar to that prevailing in the sporeling (Cutter, 1955). While the existing leaves certainly seem to affect the development of the younger organs in some way, much work remains to be done on this interesting aspect of ontogenetic development.

The time required for different species of *Nymphaea* to reach the flowering stage is evidently very variable; thus *N. stellata* may flower 12 weeks, and *N. rubra* and *N. lotus* 15-16 weeks, after sowing, while other species do not attain this condition before their third year of growth (Müntzing, 1936). In *Nuphar lutea* and *Nymphaea alba* the plants do not flower until they are 3 years old; under favourable conditions flowering is subsequently annual (Heslop-Harrison, 1955a, 1955b). The causes of flowering in these species seem to have been little investigated. Grainger (1947) states that *Nuphar lutea* apparently forms flower initials only if floating leaves are present, but Arber (1920) has reported the flowering of plants with only submerged leaves during a summer of brilliant sunshine. The gradual accumulation of unexpanded organs around the shoot apex is of particular interest with regard to flower formation in these species. Grainger (1947) has pointed out that in *Nuphar lutea* a dormant period intervenes between the initiation of flowers and their emergence; he considers, however, that flower primordia formed during one summer may emerge as blooms in the following spring. Raciborski (1894a) states that in both *Nuphar* and *Nymphaea* the inner flower primordia are likely to bloom in the second year. The present observations suggest, however, that the youngest flower primordia present probably will not come to anthesis until about 3 years later.

In both *Nuphar lutea* and *Nymphaea alba* flower primordia are somewhat larger than leaf primordia at their inception, yet, in *Nuphar* at least, the peduncle scars are considerably smaller than those of the petioles at maturity, the difference in size being sometimes sufficient to disrupt the smoothness of the parastichy rows to some extent. It seems probable that this change in the size relationship is not wholly due to the absence of marginal growth in the flower primordium. It would be of interest also to discover whether the inception of flower primordia is correlated with any change in the length of the plastochrone.

The literature suggests that other genera of the Nymphaeaceae are also of special morphogenetic interest. It is already evident, however, that *Nuphar* and *Nymphaea* afford abundant scope for studies of morphogenesis, the results of which will be set out in further contributions to this series.

Summary

The morphology of the two common British species of water-lilies, *Nymphaea alba* and *Nuphar lutea*, is described and discussed with a view to outlining morphogenetic problems within the Nymphaeaceae. The homology between leaves and flowers which has long been known has been shown to extend, in *Nymphaea*, also to vegetative buds, which occupy leaf sites in the genetic spiral. In *Nuphar lutea* vegetative buds are axillary in position; they occur in positions where flowers would be expected and always occupy the site of the first (oldest) of a group of flowers.

The morphological organization of the shoot apices of these species is also described. The young primordia of leaves and flowers are spirally arranged around the shoot apex, and are not in contact. It is shown that during ontogeny various changes take place; there is a considerable increase in size of the shoot apex, a decrease in the size of the young primordia relative to the shoot apex, and a progressive accumulation of unexpanded leaves and flowers around the shoot apex. Both leaves and flowers probably do not expand until 3 or even 4 years after their inception. Similarities between the apical organization of these plants and that of some ferns are discussed.

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STUDIES OF MORPHOGENESIS IN THE NYMPHAEACEAE. II — FLORAL DEVELOPMENT IN *NUPHAR* AND *NYMPHAEA*: BRACTS AND CALYX

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Introduction

An important aspect of morphogenetic studies is the appraisal of developmental differences between species and the determination of the stage at which these differences first become manifest. Furthermore, it is important to elucidate the growth relationships, themselves under genetic control, which bring about these developmental differences. A comparative examination of the young developing flowers of *Nymphaea alba* L. and of *Nuphar lutea* (L.). Sm. and *Nuphar advena* Ait. reveals certain relationships in the early stages of flower ontogeny in these species which apparently have not so far been reported. These observations may be of some importance both as an example of the early manifestation of genetic differences, and in clarifying certain controversial issues in the earlier literature concerning these species.

The position of the flowers in some members of the Nymphaeaceae was formerly the subject of considerable discussion and some controversy. Payer (1857) regarded the flower of *Nymphaea alba* as being formed in the axil of a leaf, which was, nevertheless, situated at some distance from it; in consequence it required some experience to find the axillant leaf. Other authors, however, recognized the extra-axillary and ebracteate nature of the flowers, which in fact originate at leaf sites in the genetic spiral (Conard, 1905; Eichler, 1878; Heslop-Harrison, 1955b; Planchon, 1853; Raciborski, 1894a; Schumann, 1890; Troll, 1937). Church (1904) admitted the absence of the bract, but supposed that an invisible growth centre was present in its place and that this occupied the leaf site in the spiral, the flower being axillary to it.

Payer (1857) was unable to recognize the developing flower[1] of *Nuphar lutea*, and thus could not say whether or not it was axillary to a leaf. Trécul (1845), however, established the presence of a scale-like bract at the base of the peduncle in this species, this being later confirmed by Raciborski (1894a). Schumann (1890) was unable to find a bract, but in view of Raciborski's findings he later (1894) suggested that he might perhaps have examined specimens of *Nuphar advena* in error. Because of the presence of this bract, the flowers of *Nuphar* are usually considered to be axillary in position (Baillon, 1872; Conard, 1905; Dutailly, 1877; Eichler, 1878; Heslop-Harrison, 1955a; Raciborski, 1894a; Troll, 1937). In his original paper on *Nuphar*, Trécul (1845) reported the occurrence of the bract, but did not actually describe the flowers as being axillary; as a result of Planchon's (1851) comments, however, he remedied this omission in a footnote to a subsequent paper (Trécul, 1854). Some other genera of this heterogeneous family are also said to possess axillary flowers (Baillon, 1872; Eichler, 1878; Li, 1955; Planchon, 1853; Raciborski, 1894b).

The absence in *Nymphaea* and *Victoria* of the bract which is present in *Nuphar* has been frequently discussed, but the origin of this organ does not seem to have been fully investigated. Trécul (1845) was uncertain whether or not it was formed before the peduncle, which, however, he

1. Payer and other authors use the term *inflorescence*; the terms *flower*, *floral axis* and *floral meristem* have been adopted in this paper.

distinguished from the basal part of the floral axis. Raciborski (1894a) stated clearly that the bract developed belatedly and that none was yet associated with the youngest flower, but he did not describe its inception. This author and others have ascribed phylogenetic significance to the presence or absence of a subtending bract, and this alone seems to indicate the need for a study of its origin and development. It seems possible, indeed, that a developmental approach may throw new light on a long-standing problem of comparative morphology.

Materials and Methods

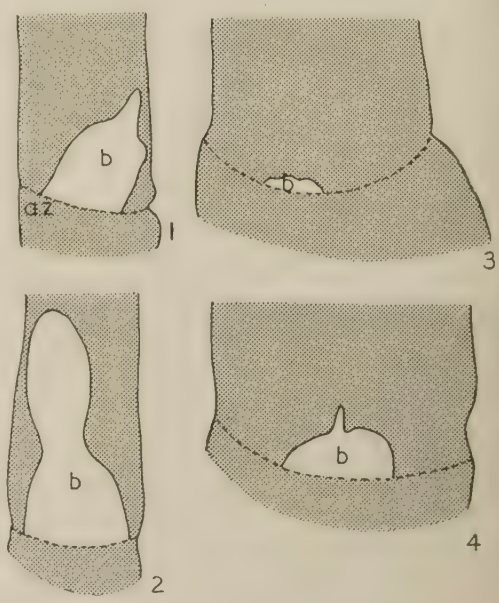
The apices of large rhizomes of *Nymphaea alba* L., *Nuphar lutea* (L.) Sm. and the North American species *Nuphar advena* Ait. were laid bare as described in a previous paper (Cutter, 1957), a number of young primordia being allowed to remain around the shoot apex. Young flowers in various stages of development were observed with a binocular microscope, and camera lucida tracings of the apex and primordia were made at a magnification of × 65. It is worth noting that young flowers were found close to the apex, i.e. in early stages of development, in the great majority of specimens of *Nuphar lutea* collected between May and November, and in those of *Nymphaea alba* collected between May and September.

Apical material for sectioning was either fixed in chromacetic solution and stained with Delafield's haematoxylin and safranin (Chamberlain, 1926), or was fixed in Craf III (Sass, 1940) and stained with safranin, orange G and tannic acid with iron alum (Sharman, 1943). The latter treatments were found to give particularly good results with material of *Nuphar* spp. Older developing flowers were fixed in formalin-acetic-alcohol (Johansen, 1940) and stained with Sharman's combination. Camera lucida tracings were made of all specimens before fixing. Serial transverse and longitudinal sections of flowers in various stages of development were cut, usually at a thickness of 10 μ. Longitudinal sections were orientated in the plane of the shoot apex, i.e. in the antero-posterior plane of the flower.

In all drawings and photographs of sections of developing flowers the subtending shoot apex is situated towards the right, i.e. the anterior face of the flower is on the left in all figures.

Bract Morphology in *Nuphar* spp.

In *Nuphar lutea*, as earlier authors have observed, a small scale-like organ is present at the base of the peduncle of mature flowers, and of flowers which will come to anthesis in the following year (Figs. 1, 5, 6a, 7). This organ is described in the literature as a bract, and accordingly this term will be retained in this paper; terminology is subsequently more fully considered. In the specimens observed in

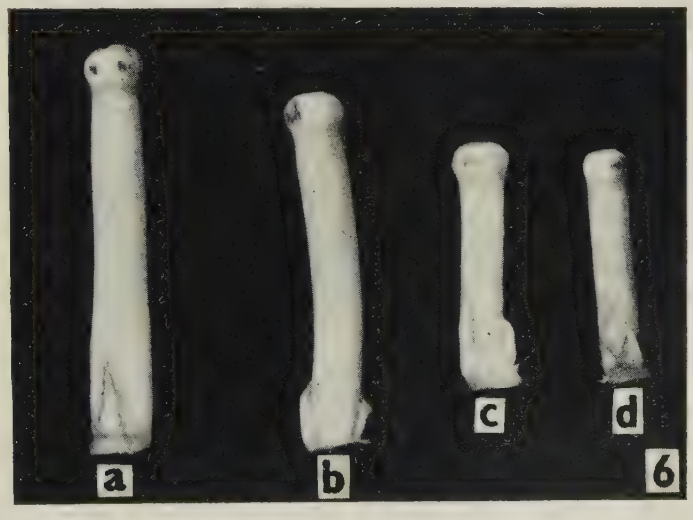


FIGS. 1-4 — Bases of peduncles of flowers of *Nuphar* spp. which will expand in the following year. The abscission zone (*a.z.*) is shown as a broken line, the tissue below this being excised from the stock. The bracts (*b*) are shown white. Fig. 1. *Nuphar lutea*. Peduncle with typical lanceolate bract. Fig. 2. *N. lutea*. Peduncle with the less common ovate type of bract. Fig. 3. *N. advena*. Peduncle with typical form of bract. Compare Fig. 1. Fig. 4. *N. advena*. Peduncle with a bract whose form resembles that of the bract in *N. lutea*. Compare Fig. 1. × 2¼.

the present work the majority of these organs were somewhat lanceolate in form, with a rather shield-shaped base (Figs. 1, 5, 7); the distal part of the bract was sometimes inrolled, giving it an awl-like form. Occasional bracts were observed in which the distal part was thicker and ovate or obovate in form, giving it a lamina-like appearance (Fig. 2). Trécul (1854) recorded the occurrence of these two forms of bract and stated that he had figured them in an album of unpublished drawings; he also reported that the oval form was the more frequent in his specimens. Vascular tissue is present in the bracts, and the branched idioblasts so characteristic of the Nymphaeaceae have been observed in the tissues of the ovate form.

It may also be noted that sometimes two of these scale-like bracts are present at the base of the mature peduncle in *Nuphar lutea* (Figs. 6b, c, d; 8-10); this fact does not seem to have been previously recorded for this genus. The arrangement of these structures was variable; they might be approximately opposite (Fig. 6 b, 8), at approximately the Fibonacci angle (Figs. 6c, 9), or almost adjacent (Figs. 6d, 10). Bracts in *N. lutea* vary somewhat in size; where two were present they were sometimes of unequal size, and that in the normal, abaxial position was not always the larger. Similarly, the two bracts were not always of the same form (Fig. 9), and this observation tends to minimize any significance which might be attached to the different frequencies of ovate or lanceolate bracts in these specimens and in those observed by Trécul (1854). The occurrence of two bracts at the base of the peduncle was not a constant feature of the flowers of a particular apex or plant. Some flowers associated with two bracts possessed 5 sepals with normal aestivation, others had 6-7 sepals with abnormal aestivation; in the latter instance the last-formed sepals were smaller than usual. Both these conditions may also be found in flowers with only one associated bract. In the specimens illustrated in Figs. 6c, d, 9 and 10, six sepals were present, their arrangement approximating to two whorls of three.

Peduncle bases and bracts of *Nuphar advena* are illustrated in Figs. 3 and 4, for comparison with those of *N. lutea* (Figs. 1 and 2). As Raciborski (1894a)



FIGS. 5, 6 — Bases of peduncles of flowers of *Nuphar lutea* which will come to anthesis in the following season. See also Figs. 7-10. Fig. 5. Typical condition, with one lanceolate bract. × 2. Fig. 6, *a*. Typical condition, with one bract *b*, *c*, *d*. Two bracts are present; in *b* they are in almost opposite positions; *c*, at approximately the Fibonacci angle; *d*, almost adjacent. × 1.

reported, the bract of *N. advena* is typically very much smaller than that of *N. lutea* and is, indeed, difficult to find, since it is obscured by hairs. Raciborski considered it to be so reduced that it could be recognized as a bract only on the grounds of its analogy with the bract in *N. lutea*, and Troll (1937) also follows this view. The typical form of the bract in *N. advena* is illustrated in Fig. 3, and a specimen in which the form of bract approaches the condition in *N. lutea* is shown in Fig. 4.

Flower Ontogeny

Examination both of the mature rhizomes of *Nymphaea alba* and *Nuphar lutea* and of the young primordia at the shoot apex shows that the flowers are formed in leaf positions in the genetic spiral (Figs. 11

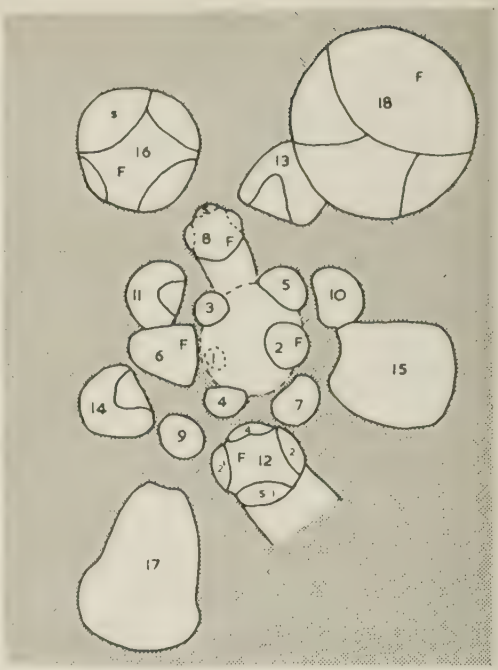


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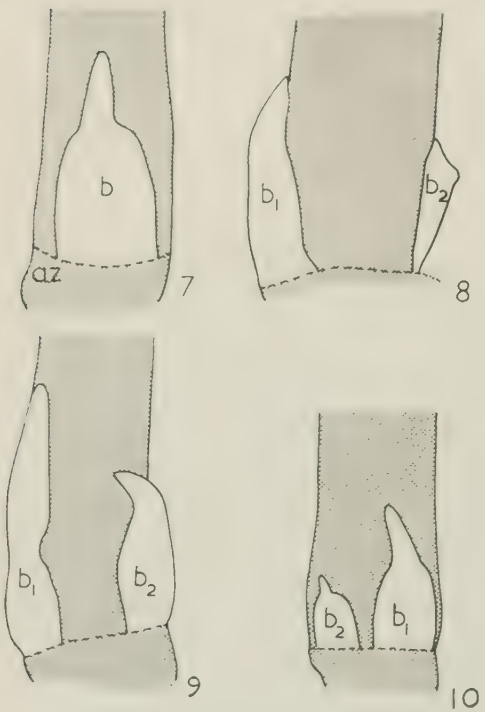
FIG. 11 — *Nymphaea alba*. Surface view of an apex in which P_2, P_6, P_8, P_{12}, P_{16} and P_{18} are young flowers (*F*). Note that while no lateral primordia are shown on the flower at P_6 (the first sepal was probably just arising), the flower at P_8, two plastochrones older, has formed three of the four sepals (*s*). On the flower at P_{12} the sepals are numbered in the order of their formation. The approximate extent of the apical meristem is indicated with a broken line; the sub-apical region is stippled. × 30.



FIGS. 7-10 — Drawings of the peduncle bases of *Nuphar lutea* illustrated in Fig. 6, *a-d*, respectively. b_1, bract in the normal abaxial position; b_2, supernumerary bract. In Fig. 9, b_1 is the ovate form of bract, while b_2 is the commoner lanceolate form. × 3.

and 12; *see* also Cutter, 1957). The morphology of the apex and young primordia of *Nuphar advena* is very similar to that of *N. lutea*. Observation of very young primordia at the shoot apex in both these species of *Nuphar* confirms Raciborski's (1894a) statement that no bract is associated with the youngest flowers. In the three species of waterlilies so far examined, it has been possible to distinguish between flower and leaf primordia at a very early stage of development, contrary to the experience of Conard (1905). Indeed, such primordia are usually distinguishable during their first plastochrone by differences in their size and shape (Cutter, 1957). At such an early stage

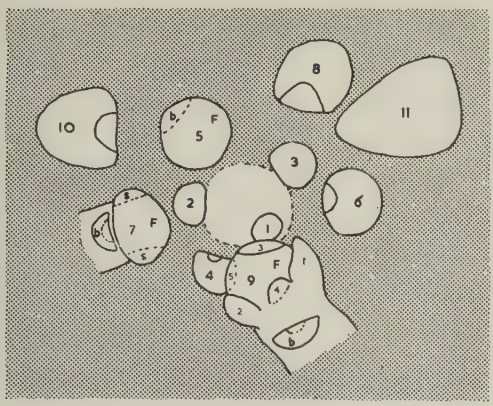
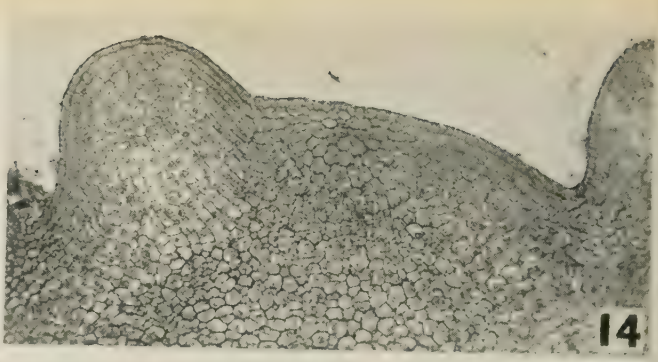


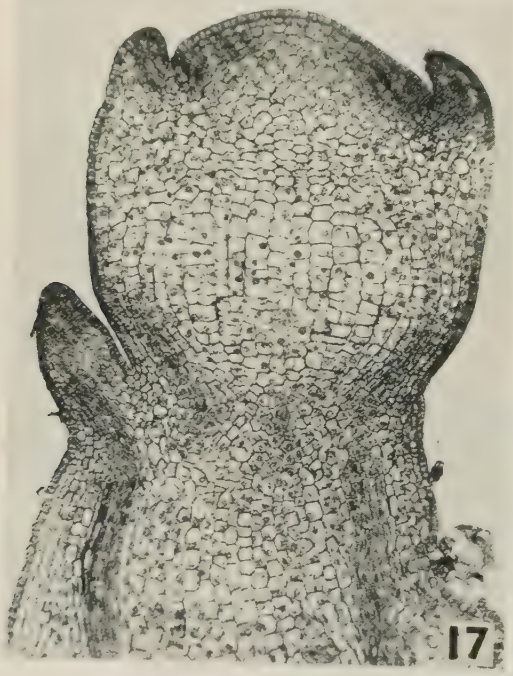
FIG. 12 — *Nuphar lutea*. Surface view of an apex in which P_5, P_7 and P_9 are developing as flowers (*F*). On the flower at P_5 the first primordium, that of the bract (*b*), has been formed. On the flower at P_7 the floral meristem has grown on and formed the two lateral sepals (*s*), leaving the bract (*b*) situated abaxially on the peduncle at a little distance from the apex of the flower. The flower at P_9 shows a still later stage of development; the five sepals are numbered in the order of their formation, showing the spiral sequence. The approximate extent of the apical meristem is indicated with a broken line; the sub-apical region is stippled. × 25.

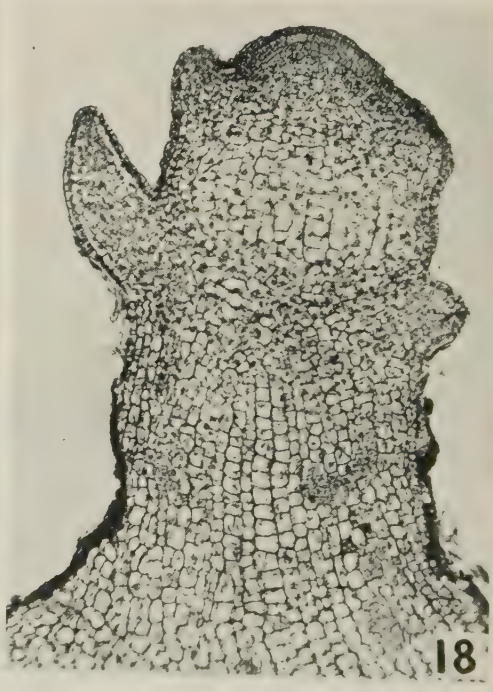
of development the young floral axes are cylindrical protuberances devoid of lateral organs (Fig. 14). Subsequently lateral primordia are formed on the floral meristems as described below. Relevant histological details will be dealt with in a subsequent paper.

As Payer (1857) and Eichler (1878) observed, the four sepals of the flowers of *Nymphaea alba* are formed in characteristic sequence: first the anterior sepal (abaxial in position relative to the main shoot apex), next the two lateral sepals, and subsequently the posterior sepal (*see* P_{12} in Fig. 11). This arrangement is said to occur also in the flowers of *Victoria* (Planchon, 1851; Schumann, 1890). In *N. alba* the first four petals alternate with the sepals.

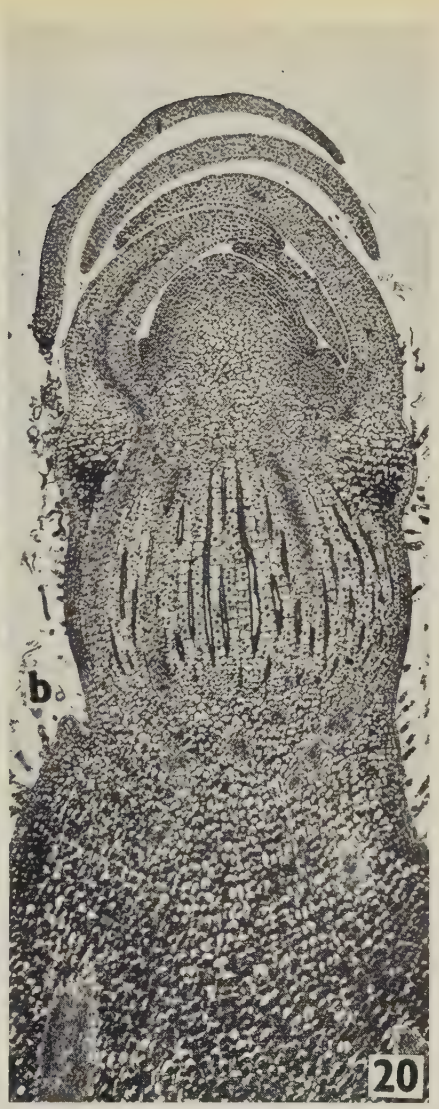
In young flowers of *Nuphar lutea* the initial sequence of organogenesis is comparable. The first primordium to be formed arises abaxially on the flower meristem (*see* P_5 in Fig. 12), the next two primordia occupy lateral positions (*see* P_7 in Fig. 12), then one posterior primordium is formed, then an anterior one, and subsequently another posterior sepal (*see* P_9 in Fig. 12, in which the 5 sepals are numbered in order of formation). These six primordia comprise the bract and the five sepals. Both external observation of the young flowers and examination of fixed material sectioned longitudinally in the antero-posterior plane of the flower show that the first, abaxial primordium of the floral meristem develops, not as a sepal, but as the bract (Figs. 15-19 and 21-25). Through the intercalary growth of the young peduncle the bract becomes progressively separated from the apex of the flower (Figs. 16-19, 23-25). Figs. 15 and 16 are particularly instructive, since they show sections of successive flowers from the same apex, separated in age by two plastochrones. A surface view of this apex was illustrated in an earlier paper (Cutter, 1957, Fig. 6). Fig. 15 is a median longitudinal section of a flower at the stage of P_5, on which the bract is just being formed; in P_7 (Fig. 16), two sepals (not in the plane of the section) are also present, and the bract primordium is now situated at some little distance from the summit of the floral meristem. From sections it is possible to trace the development of the flower from before the inception of the bract to a stage at which the latter is seen as a small scale-like organ situated abaxially on the peduncle below the region of elongation. Selected examples from this series are illustrated in Figs. 14-19 and 21-25. Prevascular tissue is present within the bract and in a path directly beneath it (Figs. 16, 17, 38). The inception of two approximately opposite bracts is shown in Fig. 18. In *Nuphar lutea*, therefore, the bract is not an organ of the main shoot apex. It is the first-formed primordium of the floral meristem and is homologous with the anterior sepal of *Nymphaea alba* (compare Figs. 13 & 15, 23 & 33). Periclinal divisions in the second layer of cells of the meristem occur in the inception of both these organs. The flowers of *Nuphar* are thus not axillary in position, but like those of *Nymphaea* are in fact formed in leaf positions in the genetic spiral.







FIGS. 13-18.



FIGS. 19, 20 — *Nuphar* spp. Fig. 19. *Nuphar lutea.* L.s. later stage in floral development, showing the position of the bract (*b*) with its prevascular trace, and its size relative to that of the sepals and petals. Fig. 20. *N. advena.* L.s. later stage in floral development, for comparison with Fig. 19. Note the extremely small size of the bract (*b*), relative both to the sepals and petals of the same flower and to the bract of *N. lutea.* Both. × 50.

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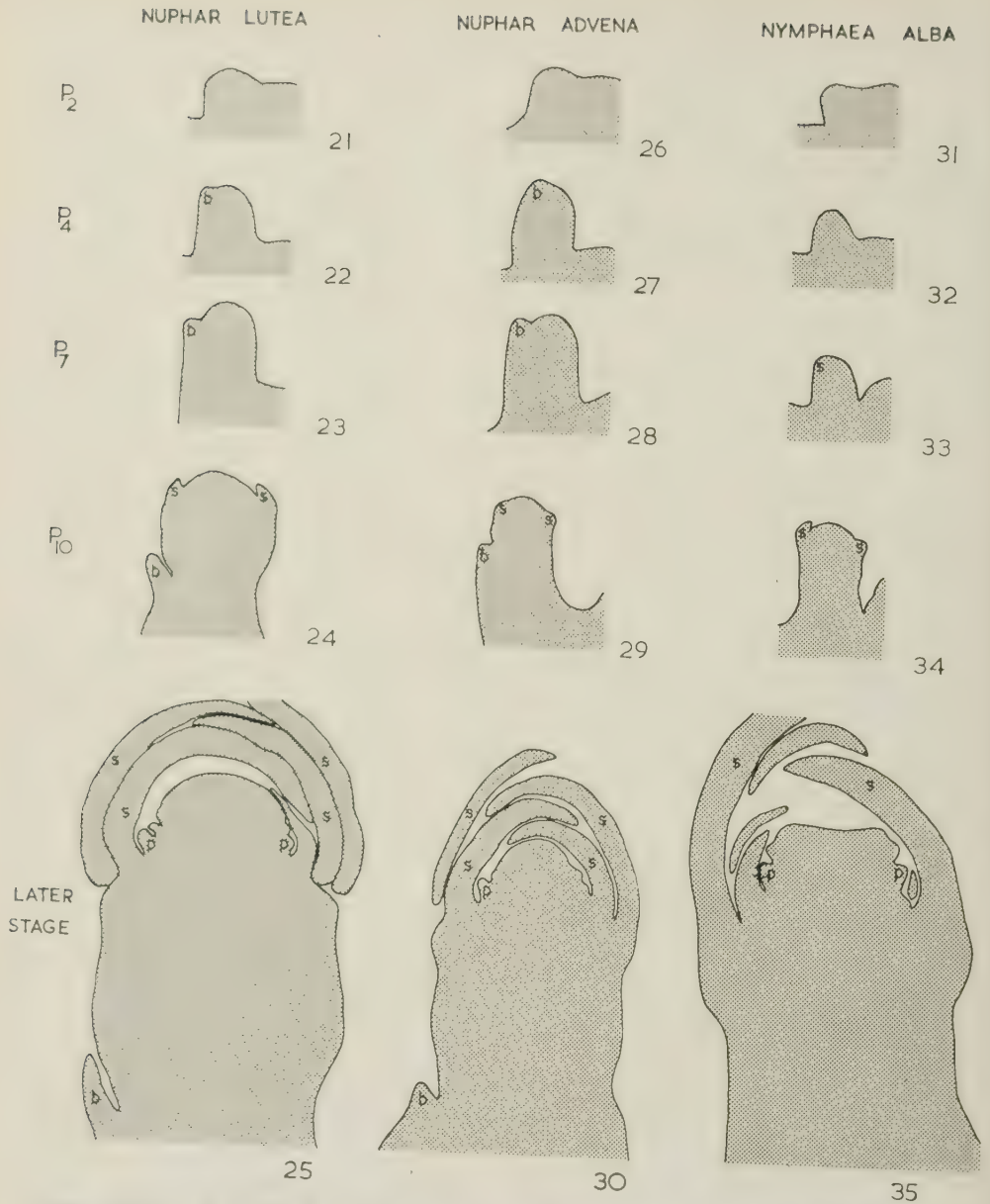
FIGS. 13-18 — Fig. 13. *Nymphaea alba.* L.s. young flower at the stage of P_6, with the anterior sepal on the left. Compare Figs. 15 and 36. Fig. 14. *Nuphar lutea.* L.s. shoot apex with young flower at P_2 (left), showing its radial symmetry and the absence of lateral organs. Fig. 15. *N. lutea.* L.s. young flower at P_5, showing the inception of the first primordium, the bract, in an anterior position. Compare Figs. 13 and 36. Fig. 16. *N. lutea.* L.s. young flower at P_7, from the same specimen as the flower shown in Fig. 15. The bract primordium with its prevascular trace is shown on the left; the two lateral sepals were also present, but are not in the plane of the section. Fig. 17. *N. lutea.* L.s. young flower at P_{10}, with bract, sepals and incipient petals. At this stage the bract is present below the elongating region of the peduncle; it is considerably larger than the fourth sepal, which is directly above it. Compare Fig. 37. Fig. 18. *N. lutea.* L.s. young flower at P_{11}, showing the inception of two approximately opposite bracts. All. × 100.



FIGS. 21-35 — (*b*, bract; *p*, petal; *s*, sepal). L.s. young flowers of *Nuphar lutea*, *N. advena* and *Nymphaea alba* in approximately comparable stages of development. The inception of the first, homologous primordium in each species is shown in Figs. 22, 27 and 33. It can be seen that this occurs at a later stage in the flowers of *Nymphaea* than in those of *Nuphar*. The bract primordium of *Nuphar advena* is initially comparable in size with that of *N. lutea* (Figs. 22, 23 and 27, 28). From about the P_{10} stage onwards (Figs. 24, 25 and 29, 30) it is clearly proportionately smaller. All. × 28.

In *Nuphar advena* the bract, at maturity, is much smaller than that of *N. lutea* (compare Figs. 1 & 7 with Fig. 3). When an apex of *N. advena* is examined under a binocular microscope, it is immediately apparent that its floral onto-

genesis is similar to that in *N. lutea*, and that the bract is formed as the first, abaxial primordium of the floral meristem. Confirmatory evidence is obtained from appropriate sections of young flowers (Figs. 27-30, 36, 37). It is noteworthy that the bract primordium is not smaller at its inception than that of *N. lutea* (Figs. 15, 36), and that the relative size of the flower and its bract remains comparable in these two species until about P_7-P_9 (Figs. 22-24, 27-29). At some stage during the subsequent development, however, the bract ceases to grow, and is later observable as a very small organ at the base of the elongating region of the peduncle (Figs. 20, 30). Prevascular tissue occurs at some distance beneath the bract primordium, but is not present within its tissues; this contrasts with the condition in *N. lutea*, in which continuous prevascular tissue is present from the tip of the bract (compare Figs. 38 and 39).

Approximately comparable stages of floral development in *Nuphar lutea*, *N. advena* and *Nymphaea alba* are illustrated in Figs. 21-35 to facilitate comparison.

Time Relationships in Floral Ontogenesis

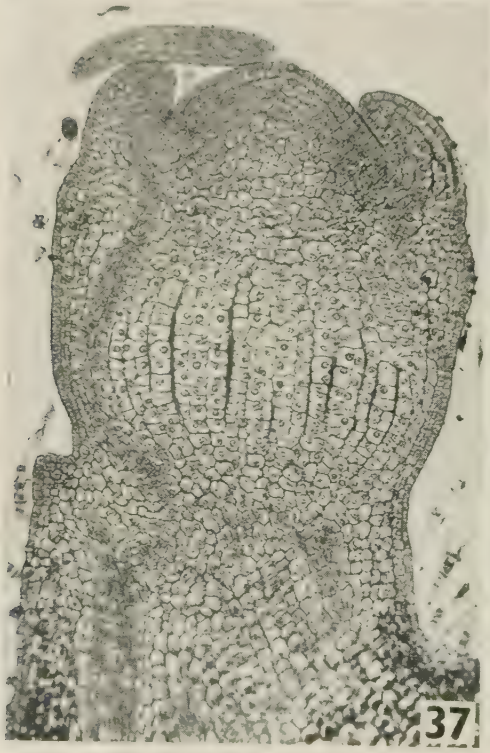
During this work the shoot apices of a number of large specimens of *Nuphar lutea* and *Nymphaea alba* were laid bare and records kept of the age in plastochrones of the young floral axes at the time of the formation of their first lateral primordia. These records, which were based on 16 apices of *Nymphaea alba*, comprising 42 flowers, and on 65 apices of *Nuphar lutea*, comprising 120 flowers, are presented graphically in Fig. 40. In *Nuphar*, the bract is the first lateral organ of the floral axis, and the presence of 6 lateral organs therefore indicates that the bract and all the calyx members have been formed. In *Nymphaea alba*, on the other hand, the presence of 6 or more lateral organs indicates that the four sepals, and usually also the first four petals (which appear to be formed almost simultaneously) have been formed.

It can be seen from the diagram that in *Nymphaea alba* all floral axes younger than P_5 were devoid of lateral primordia: the inception of the first sepal usually took place at the stage of P_5-P_7. The inception of the lateral sepals followed rapidly, and all four sepals were usually present by P_8 or P_9. The inception of the first four petals was usually accomplished at P_{10}, or sometimes even earlier. In the time relationships of these ontogenetic developments there was slight variation between different specimens, this being more pronounced in *Nymphaea*, the records for which were based on fewer specimens.

In all floral axes of *Nuphar lutea* younger than P_3 lateral primordia had not yet been formed. The inception of the bract usually took place at P_4 or P_5, and occasionally as early as P_3. Inception of the lateral sepals, however, did not usually occur before P_7, and might take place still later. Sepal formation usually was not completed until at least P_{11}. Observation of 12 apices of *Nuphar advena* yielded essentially similar records. Thus, in the floral meristems of *Nuphar* spp., the inception of lateral primordia begins earlier than in *Nymphaea alba* (Fig. 40), but some time elapses between the formation of the first and subsequent primordia, and some elongation of the floral axis takes place during this time. Indeed, in *Nuphar* the intercalary meristem which brings about the elongation of the peduncle is formed between the first primordium, i.e. the bract, and the sepals, thus bringing about their eventual separation (Figs. 15-19), whereas in *Nymphaea alba* it is formed below the insertion of the youngest primordium. In the young flowers of *N. alba*, in contrast to the condition in *Nuphar*, successive primordia are formed rapidly on the meristem (Fig. 40), and internodal development is limited, as in many other floral axes (Arber, 1937; Engard, 1944). Confirmatory evidence of these temporal relationships was obtained from sectioned materials of these species.

Spatial Relationships in Floral Ontogenesis

The sepals of *Nuphar lutea* are formed in spiral sequence, the two lateral



FIGS. 36-39.

primordia of the flower being the first sepals, as Trécul (1845) and Eichler (1878) showed. The latter described this as a characteristic orientation which he had not previously encountered elsewhere; however, it can now be seen that if the bract is included as the first primordium of the flower, the arrangement resembles, though it is not identical with, that in the flowers of *Nymphaea* and *Victoria*. In Figs. 41 and 42 the arrangement of the bract and sepals of *Nuphar lutea*, and of the sepals of *Nymphaea alba*, is presented in diagrammatic form for ease of comparison, the organs being numbered in the order of their formation. In both species, as already described, the first lateral organ (1) is formed in the anterior position on the floral meristem, but in *Nymphaea* this develops as a sepal and in *Nuphar* as a bract. In *Nymphaea* the second lateral member is not formed opposite to the first, i.e. in the posterior position, as might be expected on a space-filling theory of phyllotaxis and as apparently does occur in the flower of *Nelumbium* (Baillon, 1871), but originates in a lateral position. Two sepal primordia (2, 2′) are in fact formed approximately simultaneously, opposite to one another. The fourth sepal occupies the posterior position. In *Nuphar lutea*, after the inception of the bract there is a lapse of time and further growth of the floral meristem before the next lateral members appear. These members, the primordia of the first sepals (2, 2′), are formed in lateral positions, but instead of being directly opposite, as in *Nymphaea*, are both somewhat deflected towards the abaxial side of the floral meristem. No doubt this is made possible by a reduction in any inhibitory effects of the bract primordium as a result of the lapse of time since its inception and its vertical distance from the floral meristem. As a consequence of the positions of the lateral sepals, a spiral arrangement is initiated (Fig. 41, primordia 4, 5, 6). These observations on the sequence of early organogenesis in the flowers of both *Nymphaea alba* and *Nuphar lutea* suggest that although the floral meristems of these species are of visible radial symmetry, there is probably initially some degree of physiological asymmetry, possibly due to their proximity to the rhizome apex.

Discussion

Because of the generality of the relationship between flowers and bracts the *absence* of a bract in *Victoria* and *Nymphaea* has long engaged the interest of morphologists. The *presence* of such a bract in *Nuphar* has resulted in the flowers of *Nuphar* being described as axillary, whereas those of the allied genus *Nymphaea* are clearly extra-axillary. The demonstration now presented that, in two species of *Nuphar*, the bract is the first primordium of the floral meristem, and that consequently the flowers are not axillary, but, like those of *Nymphaea*, occur in leaf positions in the genetic spiral, has resolved this difficulty. In both these genera the young floral axes and leaf primordia are thus homologous in respect of their position and mode of origin on the rhizome apex. In subsequent studies an attempt will be made to

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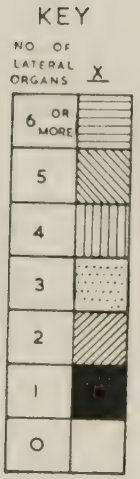
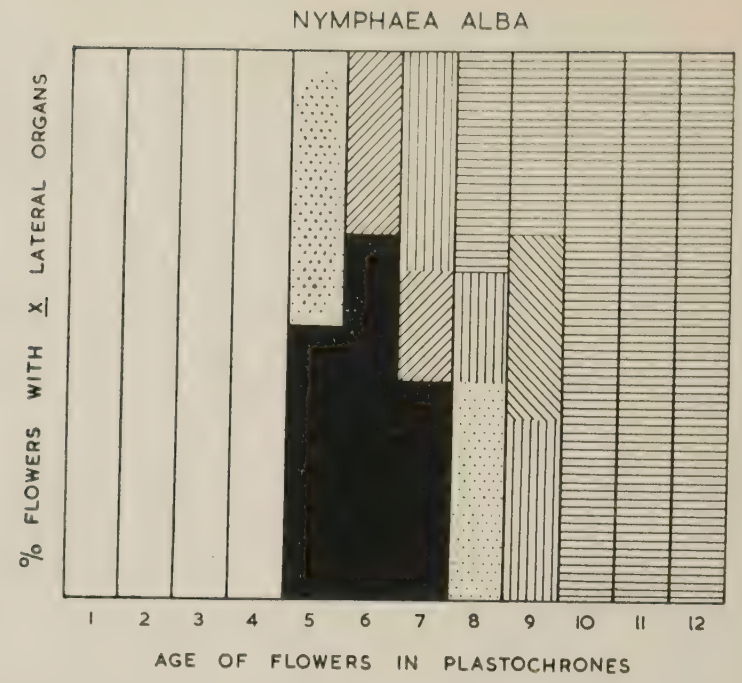
FIGS. 36-39 — Fig. 36. *Nuphar advena*. L.s. young flower at P_5, showing the inception of the bract primordium. Compare Figs. 13 and 15. × 100. Fig. 37. *N. advena*. L.s. young flower in a later stage of development, with bract, sepals and incipient petals, showing the extremely slight degree of development of the bract, which is considerably smaller than the primordium of the fourth sepal directly above it. Compare Fig. 17. × 100. Fig. 38. *N. lutea*. L.s. bract primordium, from the flower shown in Fig. 17, showing the well developed prevascular trace which is differentiated within the tissues of the bract and in a continuous path beneath it. Compare Fig. 39. × 250. Fig. 39. *N. advena*. L.s. bract primordium, from the flower shown in Fig. 37. Although from a flower in a very slightly later stage of development (compare Figs. 17 and 37) than the bract of *N. lutea* shown in Fig. 38, the bract is clearly much smaller and less developed. Note that the prevascular trace is differentiated beneath the bract, but that no prevascular tissue is present in the tissues of the bract itself. Compare Fig. 38. × 250.


KEY
NO OF LATERAL ORGANS X
6 OR MORE
5
4
3
2
1
0


NYMPHAEA ALBA
% FLOWERS WITH X LATERAL ORGANS
1 2 3 4 5 6 7 8 9 10 11 12
AGE OF FLOWERS IN PLASTOCHRONES

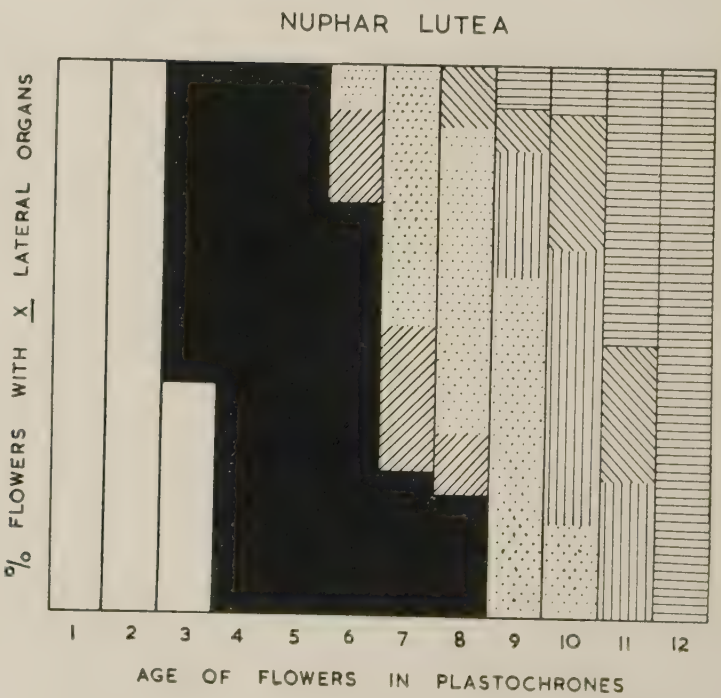
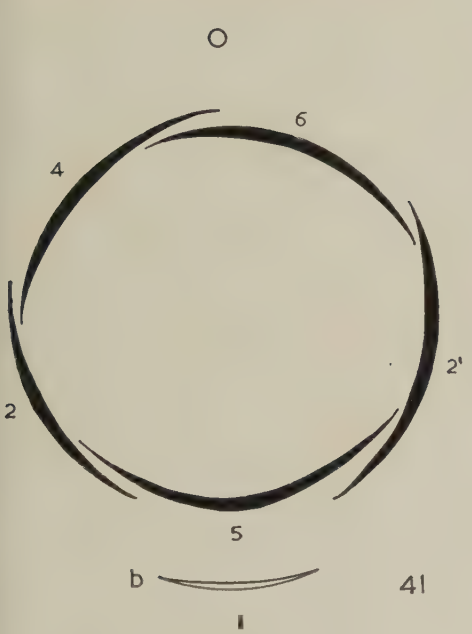
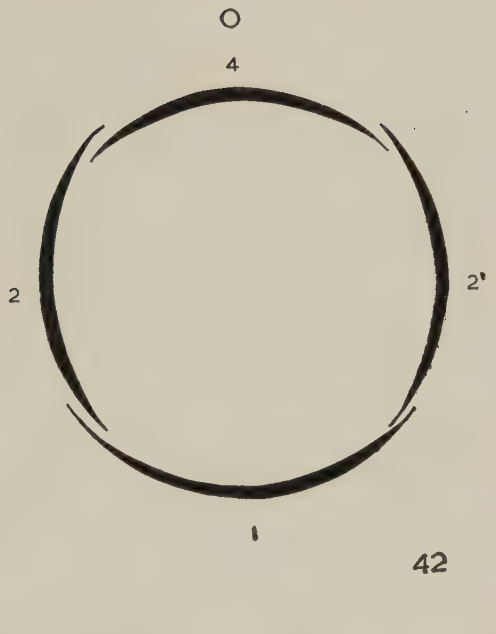

NUPHAR LUTEA
% FLOWERS WITH X LATERAL ORGANS
1 2 3 4 5 6 7 8 9 10 11 12
AGE OF FLOWERS IN PLASTOCHRONES

FIG. 40.





FIGS. 41-42 — Floral diagrams showing the arrangement of the sepals with respect to the main axis. The lateral members of the floral axes are numbered in the order of their formation. Fig. 41. *Nuphar lutea.* The bract (*b*) is the first primordium of the floral meristem, and there are five sepals (2-6). Fig. 42. *Nymphaea alba.* The four sepals (1-4) are shown. See the text for discussion.

discover the factors which determine their very different further development. Trécul (1845), who first demonstrated the presence of a bract in *Nuphar lutea*, was uncertain whether or not it was formed before the peduncle, but he regarded the basal part of the floral axis as '*tige*', the '*pédoncule*' appearing a little later at the summit of this outgrowth, from which it was at first scarcely distinguishable. Sections of young flowers yield no evidence of any distinction between these regions (Figs. 15-17); they show that an organized apical meristem is present in the young flower from its earliest stages (Fig. 14), thus indicating histogenetic continuity. Trécul's distinction was probably based on the differences in width which occur in older flowers above and below the small-celled region which is present just above the level of the bract. His illustrations (Trécul, 1845, Pl. 13, figs. 31 and 32) show the presence of the scale-like bract in this position. However, he too

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FIG. 40 — Diagram showing the relative times of inception of the lateral primordia in the flowers of *Nymphaea alba* and *Nuphar lutea*. The diagram for *Nymphaea* is based on records from 16 specimens, comprising 42 flowers; that for *Nuphar* is based on 65 specimens, comprising 120 flowers. The bract of *Nuphar* is here included as the first primordium of the floral meristem. It can be seen that the inception of primordia begins at a later stage of flower development in *Nymphaea* than in *Nuphar*, and that thereafter the inception of subsequent primordia is relatively rapid. In the flowers of *Nuphar lutea* only one primordium is present on the floral meristems over a considerable period of time, i.e. there is a lapse of time between the inception of the bract and that of the sepals.

considered the flowers to be axillary in position (Trécul, 1854).

The bractless flowers of *Nymphaea* have aroused much controversy and speculation. In the literature two different interpretations of this phenomenon can be distinguished. Several authors subscribe to the view that the bract in *Nymphaea* has aborted; that is, that the ' rudimentary ' bract which is present in *Nuphar* has completely failed to develop in *Nymphaea* [Church (1904), Planchon (1851, 1853), Raciborski (1894a), Troll (1937)]. Raciborski, who noted that no bract was associated with the youngest flowers of *Nuphar lutea*, explained this on the grounds that rudimentary organs often develop belatedly, and he derived a phylogenetic series from the ' rudimentary ' bract of *N. lutea* and *N. affine*, through the still more rudimentary swelling on the base of the peduncle in *N. advena*, to the bractless flowers of *Nymphaea*. The present demonstration that the sequence of organogenesis in the flower buds of *Nymphaea* and two species of *Nuphar* is initially comparable, and that the bract of *Nuphar* is homologous with the anterior sepal of *Nymphaea*, renders an interpretation based on the abortion of the bract in *Nymphaea* untenable. These findings, of course, do not preclude the possibility that an evolutionary series of the kind postulated by Raciborski (1894a) may exist, but they do indicate that his sequence is based on an incomplete observation leading to a false premise, and that explanations of the developments under consideration should be sought in the floral meristem itself.

According to Caspary, on the other hand, both a bract and two bracteoles are present in the flowers of *Nymphaea*, but these are normally so displaced that they occupy the positions of the anterior and lateral sepals respectively. This argument, as Schumann (1890) cogently remarked, was no doubt derived from the compelling desire of the comparative morphologists of that time to provide this anomalous flower with a subtending leaf. Caspary, who communicated his views privately to Eichler (1878), but also stated them briefly elsewhere (Caspary, 1891), had found abnormal specimens of *Nymphaea caerulea* and several other species of *Nymphaea* in which the anterior sepal was separated from the flower and inserted at the base of the peduncle; sometimes the two lateral sepals also were thus displaced. These three organs had a ribbon-like form, and the former was sometimes foliaceous. On the basis of these observations, Caspary interpreted the four sepals of a normal *Nymphaea* flower as a bract and two bracteoles which had been carried up on the flower, and a single posterior sepal. His views were endorsed by Braun, also in a private communication to Eichler (1878), and later by Conard (1905) and Worsdell (1915-16). Planchon (1851) also described specimens of *Nymphaea caerulea* in which three ' spathulate bracts ' were present at the base of the peduncle. On the basis both of his description and his illustration of such a specimen (Pl. III, Fig. 24), these organs seem to have been markedly sepaloid in form. Glück's (1924) description of a specimen of *Nymphaea alba* var. *minor* with three spathulate leaflets, which he considered to be sepals, at the base of the flower stalk and an abnormal arrangement of the calyx, is also of considerable interest. Conard (1905), who was at first sceptical of Caspary's views, was led to support them by his observations of young flowers of *Nymphaea lotus*, in which the anterior and lateral sepals were formed considerably earlier than the posterior sepal. The anterior sepal was also much larger and inserted on the peduncle much lower than the other sepals. Thus, except for the observations of Conard, the evidence supporting Caspary's views has hitherto been based on anomalous specimens. In view of the findings on *Nuphar* now reported, a re-examination of the young developing flowers of *Nymphaea lotus* would clearly be of interest. It may be noted here that the homology drawn by Conard (1905) between the five sepals of *Nuphar*, and the posterior sepal together with the first four petals of *Nymphaea*, is untenable, since it is based on the false assumption that the first sepal of *Nuphar* is posterior in position.

The present work supports Caspary's interpretation to the extent of demon-

strating that the anterior sepal of *Nymphaea* is indeed homologous with the bract of *Nuphar*. However, in terms of formal morphology it seems no more logical to regard the anterior sepal of *Nymphaea* as a modification of the bract of *Nuphar* than to take the converse view. Such considerations, moreover, seem unlikely to lead to new advances. An alternative approach is to seek the causes that underlie these differences in development. The abnormalities in *Nymphaea* spp. observed by Caspary and Planchon resemble the normal condition in *Nuphar* as described in this paper, i.e. one or more of the lateral organs formed by the floral meristem come to be situated at the base of the peduncle. In view of the evidence discussed in this paper, the *position* of the organs on the floral axis is evidently explicable in terms of the relative times of inception of (i) the first primordia on the flower bud and (ii) the intercalary meristem in the peduncle. Thus in the occasional abnormal specimens of *Nymphaea*, 1-3 primordia must have been formed on the floral meristem unusually early, before the formation of the intercalary meristem. In the specimens of *Nymphaea* observed by Caspary, only the posterior sepal was left at the top of the peduncle, the first four petals also being sepaloid (Worsdell, 1915-16). In the specimens of *Nuphar lutea* described here, however, in which two bracts were present at the base of the peduncle, the number of sepals was never less than the normal complement of five. Since it has been shown that bracts are lateral products of the flower apex, the occurrence of supernumerary bracts in this species may perhaps be regarded as a manifestation of the meristic variation known to occur in the Nymphaeaceae, although variation in sepal number is apparently relatively infrequent in *Nuphar lutea* (Heslop-Harrison, 1953a). The causes underlying such variations in the number of parts, however, still require elucidation.

It seems unlikely that the difference in *development* between two sets of homologous organs, namely (i) the bract of *Nuphar* and the anterior sepal of *Nymphaea*, and (ii) the bract of *Nuphar* and the succeeding sepals of the same flower, can be wholly due to a difference in position. This may be a contributory factor, however, since the nutrition available to a primordium which becomes progressively separated from the floral apex is likely to differ from that available to a primordium which develops in close proximity to it. It is important to note three facts concerning the bract in *Nuphar*: (*a*) its inception usually takes place at an earlier stage of development of the floral apex than that of the corresponding sepal in the flower of *Nymphaea*; (*b*) there is a lag period between the inception of the bract and that of the lateral sepals which succeed it (Fig. 40); (*c*) the bract primordium is not smaller at its inception than those of the sepals, but it undergoes only a limited further development. It may be noted that the scale leaves of some parasitic and saprophytic angiosperms, and of *Psilotum* and *Tmesipteris*, are also of normal size at their inception but fail to develop to any considerable extent (Cutter, 1955; Wardlaw, 1957a). With the bract of *Nuphar*, therefore, as with these other examples, it is the limited further development of the primordium that requires explanation.

Wardlaw (1957b) has recently suggested that conceptions relating to the heteroblastic development of leaves may also apply to the flowering apex, and since this phenomenon is also concerned with differences in the subsequent development of similar primordia, it may be that some explanation of the limited development of the bract along these lines might prove valid. Allsopp (1954) considers that the nutritional factors which have been shown to be important in heteroblastic leaf development probably act through their effect on the shoot apex. Thus the inception of the bract primordium on the floral meristem of *Nuphar* at an early stage of its development, when the nutritional status of the apex may be restricted in some way, might contribute to the limited subsequent development of this organ. The apparent correlation between the number and size of the floral parts of populations of *Nymphaea alba* and the richness of their habitat (Heslop-Harrison, 1953b) indicates that the nutrition avail-

able to the floral meristem may indeed be of some importance in floral organogenesis. It is interesting in this connection to note that the sections of *Nuphar advena* examined suggested that bract inception in this species, in which the development of the bract is extremely limited, might occur at a still earlier stage of floral development than in *N. lutea*; however, insufficient material was examined to establish this point. The poor growth potentiality of the bract primordium in *N. advena* is further indicated by the failure of the prevascular trace to be differentiated in its tissues (Fig. 39). The differences in development between the bract and the subsequently formed sepals of the same flower may perhaps be explicable in terms of the changes in physiological and nutritional conditions in the floral apex during the lapse of time between bract and sepal inception.

It is now clear that the organ which has been described as a bract in *Nuphar* is in fact an organ of limited growth formed in an abaxial position on the floral meristem; it is *not* a foliar member formed on the main rhizome and subtending a flower in its axil. A recent discussion of terminology has emphasized the diverse uses of the term ' bract ' throughout the literature (Rickett, 1954). Rickett suggests that in current usage the term merely implies a leaf-like organ which differs from the foliage leaves and is associated with flowers; clearly the bract of *Nuphar* would conform to such a definition. Notwithstanding certain connotations which imply that it subtends a flower in its axil, it therefore seems best to retain the term ' bract ' for the first lateral organ of the floral meristem of *Nuphar*. The affinities of bracts with both foliage leaves and sepals have been frequently discussed (e.g. Arber, 1937; Eames, 1931; Tepfer, 1953). The foliar characteristics of the bract of *Nuphar lutea* may perhaps suggest that in this species the floral meristem might conceivably possess some initial potentiality for vegetative development. The occurrence of lateral vegetative buds in prospective flower sites in both *Nymphaea* and *Nuphar* (Cutter, 1957) tends to support this conception. It will be important to investigate experimentally the growth potentialities of floral meristems in these species.

Finally, it should be stated that the differences in floral ontogeny which have been described in these three species of water-lilies must ultimately be determined by genetic factors. Indeed, they constitute a good example of the early expression in the apical meristem of genetic differences in related plants.

Summary

Early stages in floral development in three species of water-lilies, *Nymphaea alba*, *Nuphar lutea* and *N. advena*, have been examined both by external observation of young flowers and in suitably orientated serial sections. It has been shown that the bract which has long been known to occur at the base of the mature peduncle of *Nuphar* spp. originates as the first primordium of the floral meristem, and is thus homologous with the anterior sepal of *Nymphaea*. The flowers of *Nuphar* are therefore not axillary in position, as has previously been supposed, but, like those of *Nymphaea*, occupy leaf sites in the genetic spiral. It is shown that the basal position of the bract is due to its inception on the floral apex at an early stage of its development, prior to the formation of the intercalary meristem in the peduncle. Temporal and spatial relationships of organogenesis in the flowers of *Nuphar* and *Nymphaea* are discussed. The occasional occurrence of two bracts at the base of the mature peduncle of *Nuphar lutea* is described. These findings are discussed in the light of the conclusions and speculations of earlier authors, and an attempt is made to indicate the kind of causal explanation that is likely to apply to the observed differences in floral development in these species.

I wish to thank Professor C. W. Wardlaw for much helpful discussion and advice during the preparation of this paper. I am greatly indebted to Mr E. E. Kemp, Curator of the Royal Botanic Garden, Edinburgh, for his kindness in sending me a generous supply of material of *Nuphar advena*, and to Mr E. Ashby and Mr G. Grange for the photographic illustrations.

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ON SOME PECULIAR FEATURES IN THE EMBRYOGENY OF *PAEONIA* L.

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Embryological data are being extensively used at present to solve phylogenetic questions in doubtful cases. On the basis of such analyses views on the genetical interrelations of some groups of angiosperms may undergo radical changes. This fact is brought out in a number of reviews (Mauritzon, 1939; Maheshwari, 1945a, b, 1946, 1950; Poddubnaja-Arnoldi, 1951; Baranov, 1955) and in many special works (Romanov, 1944; Yakovlev, 1950; Lebégue, 1952; Gerassimova-Nawashina, 1954; Souèges, 1954; etc.).

The problem of the origin of the angiosperms is closely connected with a knowledge of the members of the order Polycarpicae, which has been placed at the base of the phylogenetic chart of the angiosperms. It is in this order that we find the most primitive families and genera whose investigation is of great interest both for taxonomists and embryologists. Therefore, the genus *Paeonia* attracted our attention.

Although peony is an ancient plant, known to people from time immemorial, and is cultivated all over the world, the literature on its morphology is very scarce and embryological data are practically absent (*see* Stern, 1946).

Recently one of us (Yakovlev, 1951) published some preliminary observations on the initial phases of embryo development in certain species of *Paeonia*. It was shown that the embryogeny of this genus differs from that of all the angiosperms examined previously. Although all the points in the development and differentiation of the embryonic structures were not clear, the peculiarity of the embryonic process was quite evident.

It was discovered that the development of the peony embryo could not be referred to any of the known embryonal types and required further cyto-embryological investigations and studies *in vivo* based on additional material collected under natural conditions.

The material used for the present investigation includes *P. anomala* and *P. moutan* grown at the Botanical Institute of the Academy of Sciences in Leningrad and *P. wittmanniana* collected in the mountains of South Osetia, Caucasus, near the alpine station of the Botanical Institute at an altitude of 1900 m.[1]

The ovules were fixed in the fluids of Nawashin, Carnoy and Maheshwari. The preparations made according to the usual cytological technique were stained with gentian-violet, Haidenhain's haematoxylin counterstained with Orange G, and Delafield's and Ehrlich's haematoxylin.

Along the ventral suture of the carpel there lie about twenty or more ovules. Although the flowers are abundantly visited by beetles, ants and other insects, not all of the ovules develop further under the conditions of Leningrad; the number of underdeveloped ovules in *P. anomala* varies from 35 to 70 per cent (*see* Fig. 1).

The ovule is crassinucellate, bitegmic. The nucellus disintegrates rather early. It persists partly at the micropylar end of the embryo sac forming a sort of a cap, but later on it collapses here too. The mature embryo sac adjoins the inner integument.

The mature female gametophyte contains a well-developed egg-apparatus, a large central nucleus and a three-celled antipodal apparatus. The egg-cell and the synergids contain characteristic vacuolated cytoplasm and distinct nuclei

1. We express our sincere thanks to E. A. Gogina who collected and fixed the material of *P. wittmanniana*.

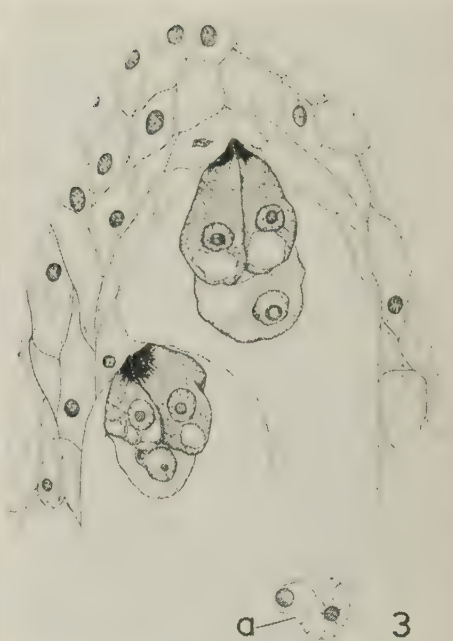
FIG. 1 — L.s. fruit of *P anomala*; note the underdeveloped ovules in addition to mature seeds.

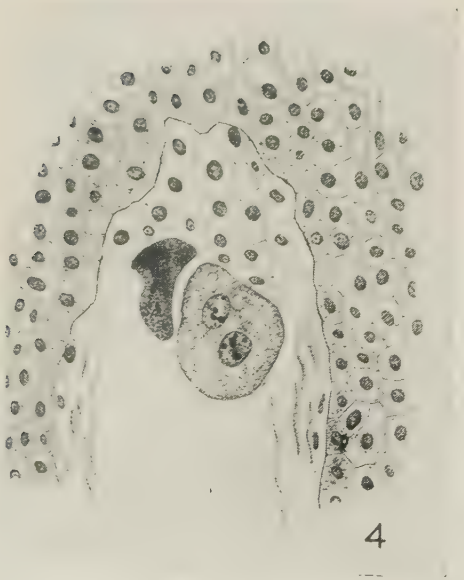
(Fig. 2). The narrower micropylar portions of the synergids have a filiform apparatus. One preparation showed the fusion of a sperm nucleus with that of the egg (Fig. 3). It is interesting to note that here syngamy is taking place under somewhat abnormal conditions, the two synergids being still well-preserved. The pollen tube has evidently entered the embryo sac without destroying any one of them.

It is known that embryo development in angiosperms is characterized by wall formation following the very first division of the zygote. This feature distinguishes the type of embryogeny met with in angiosperms from that in the gymnosperms. The difference between the embryonal types among angiosperms lies in the direction of the first and subsequent walls laid down during the process of cleavage, and in the part taken by the blastomeres in the development of the parts of the embryo. In *Paeonia*, on the other hand, karyokinesis is not followed by cytokinesis and gives rise to a binucleate cell (Fig. 4). The cytoplasm of this cell is more or less uniformly vacuolated and its nuclei contain many chromocentres. The second division soon follows, and Figs. 5 and 6 show a normal karyokinesis with synchronously dividing nuclei. Some preparations showed a granular pathway in the middle of the phragmoplast which looks like the rudiment of a cell-plate, but in fact no cell-plate is formed and we obtain instead a four-nucleate coenocyte (Fig. 7). At this stage also the nuclei contain numerous chromocentres and frequently have an irregular outline. Fig. 8 shows one of the many instances of polyembryony. Two zygotes began to develop simultaneously, evidently from two embryo sacs. One of the zygotes contains nuclei at prophase, the other at early anaphase.

The next division results in eight nuclei occupying the periphery of a peculiar proembryonic coenocyte (Fig. 9). Concomitant with the growth of the coenocyte and an increase in the number of its nuclei there is also an increase in the vacuolization of its cytoplasm. Finally the enlarging vacuoles fuse together to form a large central vacuole (Fig. 10). The nuclei are evenly distributed along the periphery being separated from one another by small vacuoles. This stage is termed by us as the "first coenocytic proembryo phase".

Without a knowledge of the history of the coenocyte it may be mistaken for one of the polynucleate structures known to occur during embryo sac and endosperm development. In *Sandoricum koetjape* (Juliano, 1934), for example, a coenocytic structure occupies a considerable space at the chalazal part of the embryo sac and appears as a second polynucleate vesicle inside the normal embryo sac. The author believes it to have originated from the fourth megaspore entering the normal embryo sac which developed from the third megaspore. As another example we may mention the helobial endosperm of *Echium plantagineum*, which shows free nuclear division and the formation of a coenocytic structure (*see* Maheshwari, 1950; fig. 141 D, F). Individualized globular structures sometimes arising in the earlier phases of nuclear endosperm





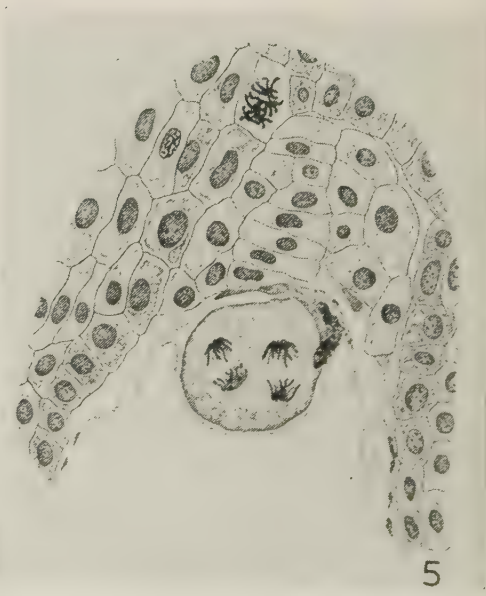
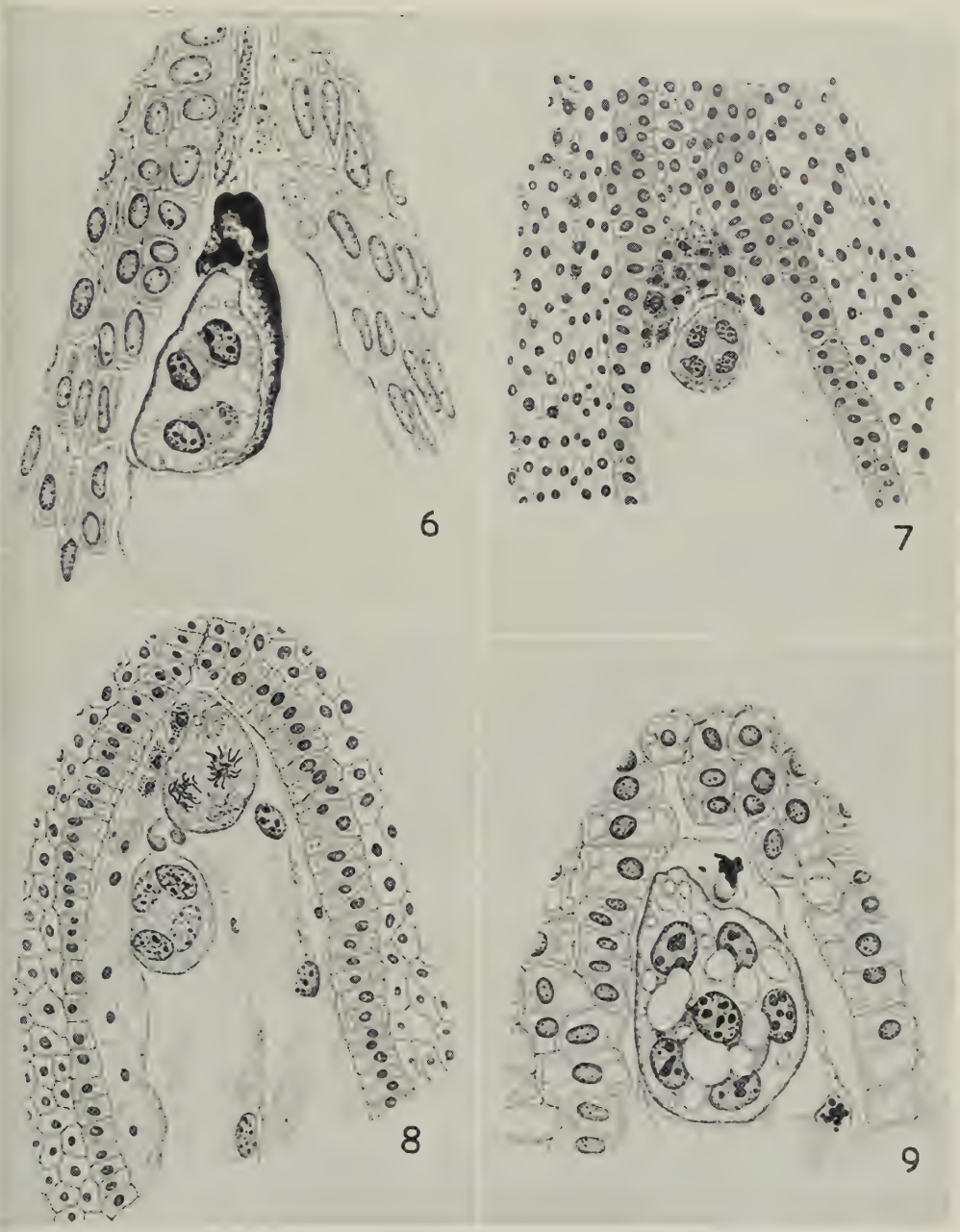
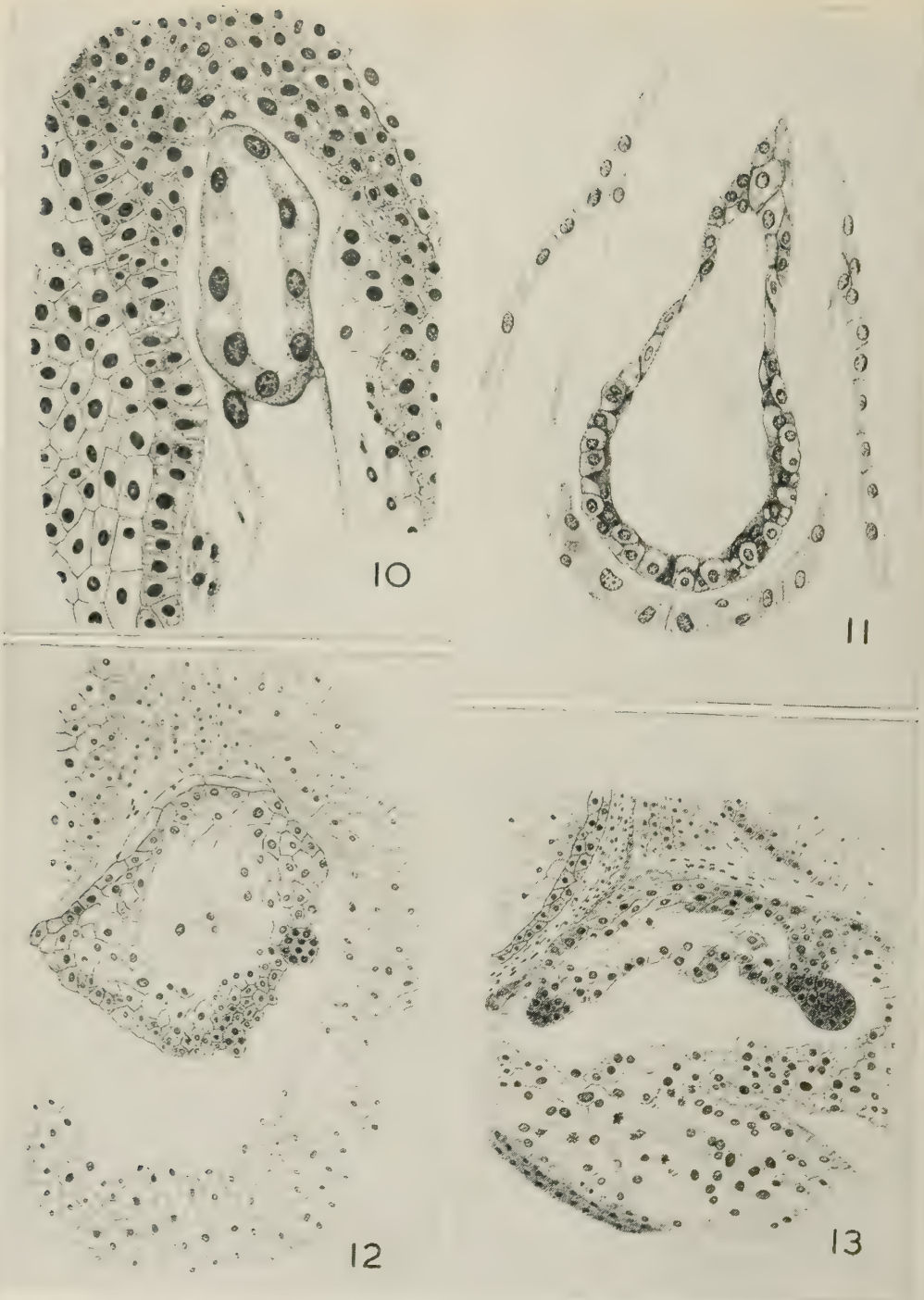


FIG. 2-5 — Fig. 2. L.s. micropylar part of embryo sac showing egg, synergids, central nucleus, nucellar remnants and epidermis of inner integument. Fig. 3. A case of twin embryo sacs with both egg apparatuses completely formed. The nucleus of the egg on left is fusing with that of sperm; same shown more highly magnified at *a*. Fig. 4. *P. wittmanniana*: l.s. micropylar part of embryo sac showing binucleate zygote, crushed synergids, nucellar remnants and inner integument. Fig. 5. Second division of nuclei in zygote; note remnants of pollen tube.



FIGS. 6-9 — Fig. 6. *P. wittmanniana*. Later stage of second nuclear division; formation of ephemeral phragmoplast. Fig. 7. Four nuclei fully formed; note absence of wall. Starch in nucellar cells and integuments is shown in black. Fig. 8. A case of polyembryony; note preparation for third division in upper embryo. Fig. 9. Eight-nucleate coenocyte formed as a result of the third division.



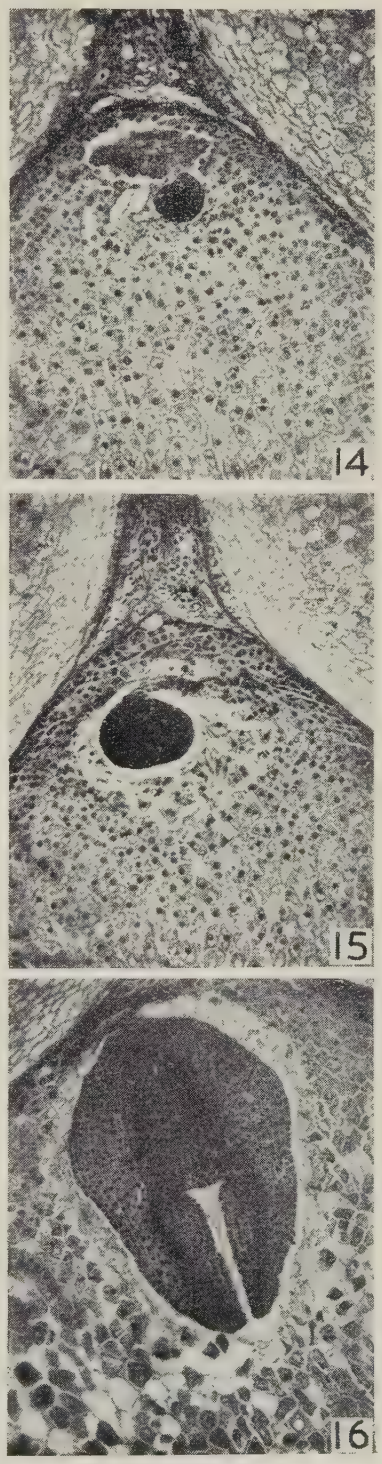
FIGS. 10-13 Fig. 10. *P. wittmanniana*: polynucleate coenocytic embryo with large central vacuole. Fig. 11. *P. anomala*: l.s. embryo sac on 14th day after anthesis showing cell formation in proembryonic coenocyte and endosperm. Fig. 12. Same, 20 days after anthesis. Two meristematic primordia have appeared from the proembryo. Note also endosperm cells with thin walls. Fig. 13. Same, 24 days after anthesis.

have been described by Juliano & Alcala (1933) in *Musa errans*.

Many examples could be cited of the coenocytic nature of certain haustorial cells in the embryo sac system. They are, however, of an entirely different origin and nature than those observed in the embryogenesis of peony. Further, they are transient and incapable of giving any new formations for they degenerate and disappear after some time. The same is not true of the coenocyte of *Paeonia*. In spite of the transitory nature of this condition it subsequently becomes a source of new viable structures composing the embryo.

The coenocytic condition lasts for a rather long time — from 12 to 14 days after anthesis — the number of nuclei gradually increasing. It must be mentioned that the endosperm presents much the same condition. The division of the secondary nucleus takes place as usual, somewhat earlier than that of the zygote and the rate of division of the endosperm nuclei exceeds that in the proembryo. Thus when the proembryonal coenocyte has two nuclei, the endosperm may contain as many as ten, and at the four-nucleate coenocyte stage, the endosperm contains about twenty nuclei. As the coenocyte assumes the form of a vesicle with a large central vacuole and peripherally distributed nuclei, the endosperm shows a similar construction. It has the character of a cytoplasmic sheath with imbedded nuclei which are rather crowded at the micropylar and chalazal ends of the sac.

The first sign of the differentiation of a proembryonic coenocyte is the appearance of cell walls between its nuclei and the formation of a peripheral cell layer (Fig. 11). As the result of further multiplication of the cells the whole coenocyte is



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FIGS. 14-16 — *P. moutan*. Fig. 14. L.s. micropylar part of embryo sac showing the collapsed proembryonic structure, developing embryo and cellular endosperm. Fig. 15. Later stage at the time of collapse of proembryonic structure. Fig. 16. More advanced stage showing mature embryo; note cotyledons, procambial strands and remnants of the flattened proembryonic structure.

converted into a cellular structure with only a small cavity left in the middle.

Cell formation does not, however, result in the direct transformation of the proembryonic coenocyte into an embryo as in the gymnosperms (*Ginkgo*). In *Paeonia* the subsequent development is also peculiar. After the proembryo assumes a cellular state, certain peripheral cells divide actively and form meristematic centres which protrude and give rise to the initials or primordia of future embryos (Fig. 12). There are generally several such protuberances but only one of them develops into an embryo, the other initials ceasing their development rather early. From the moment the embryo primordia are laid down, and till the complete formation of the embryo, the latter remains connected with the proembryonal structure which has produced it (Figs. 12-16). Fig. 10 shows the turgescent character of the structure in question. Fig. 13 demonstrates that as the embryonic protuberance continues to grow, the original coenocytic structure loses its turgor and begins to collapse. Figs. 14-16 show the further development of the embryo. It is evident that the proembryonic tissue not only gives rise to embryos but also nourishes them during their growth.

Discussion

Generally the development of the embryo begins with an initial cell, the zygote. The embryo of angiosperms, though very diversified, begins its development with cell divisions followed by an early cellular differentiation. The very first two blastomeres arising from the zygote differ functionally and represent a polarized system. The successive divisions maintain a continuity in the general course of embryonal development and lead to a more profound differentiation.

In peony the situation is different. By free-nuclear divisions, unaccompanied by cell formation, the zygote turns into a polynucleate coenocyte. This condition underlies the development of the coenocyte as an individualized structure and makes the early differentiation of embryonic structures impossible.

The coenocytic condition is considered by us as the first stage preceeding the localization and differentiation of embryonic structures which occur only at the ensuing, second stage. A number of other stages can also be distinguished. Thus the appearance of a central vacuole in the coenocyte should be regarded as changing the morphological appearance and physiological properties of the polynucleate coenocyte. Cell formation in a coenocyte is also important but we wish to concentrate our attention upon two main stages — the proembryonic and the embryonic proper.

It is difficult to say what is the cause of such a peculiar two-step embryogenesis as in *Paeonia*. Only some general suggestions may be put forward, which demand verification not only in regard to peony but also in regard to other members of higher plants. The peculiar type of embryogeny described for peony broadens our concept of the embryonic process and leads to the conclusion that a zygote does not in every case become a direct initial of an embryo and that the embryonic process in angiosperms does not always begin with cell formation but in some instances may include a coenocytic phase as well.

Polynucleate cell formation is not uncommon in plants. In some cases it is a pathological phenomenon caused by external stimuli, such as insect punctures; in others it is an element of a normal cycle of certain cell structures, e.g. tapetal cells, different haustoria, etc. Such polynucleate cells are of a specialized character and break down after fulfilling their function. The development of the female gametophyte and giant pollen grains (the so-called Němec phenomenon) are other examples of a similar nature. In these cases the coenocytic structure results in the formation of specialized cells which are normally incapable of autonomous development.

The question arises whether such a coenocytic structure may also be formed during embryogenesis.

A coenocytic embryo was reported by Rutgers (1923) in *Moringa oleifera*. According to him cell formation begins only after the 16-nucleate stage. This

was, however, contradicted by Puri (1941) who showed the coenocyte to be merely a micropylar accumulation of endosperm nuclei. A case of coenocyte formation was also recorded by Tischler (1913) in the parthenogenetic development of the egg of *Ficus*, but the author considered this to be an anomaly. A similar view was expressed by Cappaletti (1929; cited from Maheshwari, 1950) who investigated the embryogeny of *Ruta*.

Definite information on possible free-nuclear divisions in the normal zygotic development of angiosperms is thus lacking. Among higher plants only gymnosperms have a coenocytic phase followed by cell formation and differentiation of embryonic structure.

The most primitive type of embryogenesis among the gymnosperms is observed in *Ginkgo* which shows a very large number of free-nuclear divisions in the coenocytic phase. In the Cycadales the number of free nuclei reaches 2^9 (512), rarely 2^{10} (1024). In the Coniferales this number is reduced to 16, 8 and 4. The embryo initial thus formed consists originally of a small number of cells (rosette cells, suspensor cells) which already have specific functions. In Gnetales the number of free nuclei is very small; in *Welwitschia* and *Gnetum* there is perhaps no free nuclear stage at all. Thus in the gymnosperms there is a clear tendency towards reduction of the number of free-nuclear divisions prior to embryo formation.

The presence of a coenocytic phase in the embryogenesis of peony made us pay special attention to this phenomenon in connection with the problem of the origin of angiosperms. Free nuclear divisions in the egg originally appeared in gymnosperms, but they already show a tendency towards reduction of the coenocytic phase. Thus in higher plants two main types of embryonic development are distinguishable. In pteridophytes only cellular structures take part in the process of embryogenesis; in gymnosperms embryo development begins with the free-nuclear divisions; and in angiosperms both these modes of development are present.

The occurrence of a coenocytic phase in the embryogenesis of Paeoniaceae is a strong point against the existence of the barrier created by taxonomists between the Angiospermae and the Gymnospermae. While emphasizing the presence of a coenocytic phase we do not, of course, completely correlate the process of embryogenesis in gymnosperms and angiosperms. Each group has its own specific characters and biological peculiarities. It does not, however, eliminate those common characteristics which bring together even such wide systematic groups.

The study of embryogenesis in Paeoniaceae will help in the solution of some complicated problems of the phylogeny and especially the question of the origin of angiosperms.

Summary

The development of the embryo of *Paeonia* is different from that which normally occurs in other angiosperms. The first division of the zygote is not followed by wall formation. Several of the subsequent divisions are also free nuclear. The nuclei become evenly distributed along the periphery while the centre is occupied by a large vacuole. This coenocytic condition is followed by cell formation which, however, does not directly result in an embryo. Instead, certain peripheral cells form meristematic centres which protrude and give rise to embryonal primordia. While there are several such protuberances, only one generally matures into an embryo.

The development recorded in *Paeonia* is compared with that in certain gymnosperms like *Ginkgo*.

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NOTES ON SOUTH AFRICAN MARINE CHLOROPHYCEAE

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The present paper deals with the identity, taxonomy and nomenclature of some of the South African marine green algae. The study was assisted by a grant-in-aid to the senior author (1954 -) from the National Science Foundation for which grateful acknowledgment is made.

Willeella ordinata Boergesen

Willeella ordinata Boergesen, 1930, p. 155, Figs. 3, 4, Pl. 1, Fig. 1.

This genus is here reported for the first time from South Africa. The specimens were collected by Papenfuss and Pocock in July 1938 at Isipingo and Port Shepstone in Natal.

Willeella has been credited with three species: *W. ordinata* Boergesen (1930), the type of the genus, *W. japonica* Yamada et Segawa (in Segawa, 1938), and *W. mexicana* Dawson (1950). The herbarium of the University of California contains isotype material of *W. ordinata* and Professor Yamada has kindly sent us a specimen of *W. japonica* that Segawa in 1942 collected at Tōzi, near Shimoda, in Izu Province. We know *W. mexicana* only from Dawson's description. Whether *W. mexicana* is different from *W. ordinata* appears doubtful. The less symmetrical branching in the plant from Mexico may be the result of injury to the thallus, regularity of growth occurring in un-

disturbed parts. All the features whereby Dawson separates *W. mexicana* from *W. ordinata* may be observed in the South African material, which, however, agrees very well with Boergesen's description of *W. ordinata* as well as the isotype of this species. The only difference between the plants from South Africa and India lies in the fact that in those from South Africa the cells in the main axes occasionally undergo intercalary transverse division and the newly formed segments in turn produce opposite branches at the distal end.

Because of its fan-like habit, Boergesen assigned *Willeella* to the Anadyomenaceae, a position also accorded the genus by Feldmann (1938). Papenfuss (1955) has transferred the Anadyomenaceae from the Siphonocladales to the Cladophorales on the basis of present knowledge of the genera *Anadyomene* and *Microdictyon*. In addition to these two genera and *Willeella*, the family Anadyomenaceae has been credited with *Valoniopsis*, *Rhipidiphyllon* and *Cystodictyon*.

As segregative division of the protoplast, a process characteristic of the Siphonocladales, has not been observed in *Willeella* by us nor (apparently) by the other investigators of the genus, we believe that the genus is correctly placed in the Anadyomenaceae and Cladophorales. It seems likely that *Rhipidiphyllon* and *Cystodictyon* are also correctly referred to the Anadyomenaceae. In our opinion *Valoniopsis* is more appropriately placed in the Valoniaceae, as will be explained farther on under that genus.

Boodlea Montagnei (Harvey ex J. E. Gray) Egerod

Boodlea Montagnei (Harvey ex J. E. Gray) Egerod, 1952, p. 332, fn.

Microdictyon Montagnei Harvey ex J. E. Gray, 1866, p. 69; Reinbold in Weber-van Bosse, 1913, p. 67; Setchell, 1926, p. 151; 1929, p. 573, Figs. 97-105; 1935, p. 131.

Boodlea paradoxa Reinbold, 1905, p. 148.

Brand in 1905 (p. 191) reported *Boodlea kaeneana* Brand from South Africa on the basis of material collected by Krauss and preserved in the herbarium of Martens. Egerod (1952, p. 362) has merged this species in *B. composita* (Harvey) Brand. We have not seen the South African material in question (if it still exists) and it is thus not known whether it actually is representative of *B. composita*, a species first described from Mauritius and known to occur in the Red Sea among other places.

This is the first report of the occurrence of *Boodlea Montagnei* in South Africa. The record is based on a single specimen which was present among formalin-preserved material of *Microdictyon Kraussii* that was collected at Umpangazi (Natal) in May 1939 by Professor T. A. Stephenson and his collaborators.

In identifying this specimen with *Boodlea Montagnei*, we were faced by two problems: (1) Does this species belong in *Boodlea* or in *Microdictyon*, in which genus it had been placed for a long time? (2) If it belongs in *Boodlea*, should it be referred to *B. Montagnei* or to *B. paradoxa*?

As has been pointed out by Reinbold (in Weber-van Bosse, 1913, p. 67) and Setchell (1926, p. 151; 1929, pp. 468, 572), both of whom regarded *Boodlea Montagnei* as a species of *Microdictyon*, this taxon differs from species of *Microdictyon* by forming special anastomosing organs (tenacula), in which respect it agrees with species of *Struvea* and *Boodlea*. Although the frond is usually two-dimensional or plane in *B. Montagnei*, there is, as Setchell (1929, p. 577) has remarked, "... a tendency to depart from the strict plane characteristic of the genus [*Microdictyon*], ..." Such a condition is illustrated by Setchell (1929, Fig. 101). Setchell (op. cit. pp. 577-578) further remarks: "The specimens from the Dutch East Indies, ..., show a seeming series of intergradations ... between *Boodleoid* Microdictyons and true *Boodlea* ... Certain fronds seem to be true *Microdictyon Montagnei* in the center and *Boodlea paradoxa* at the margins. This seems to indicate the possibility of *Boodlea paradoxa* being only a state of *Microdictyon Montagnei*." In a later paper, after having seen the species growing in quantity along the shores of the Island of Bali, Setchell (1935, p. 131) wrote: "It seems more and more plausible that the *Boodlea paradoxa* Reinbold ... is only a condition of *Microdictyon Montagnei* Harv... From

the various collections now available, it seems fairly clearly indicated that in quiet spots (or whole habitats) the flat expansion of *Microdictyon* is produced, but in boiling surge or even moderately rough water the spongiose *Boodlea* habit is partly or completely assumed."

It is evident, then, that on habit alone, *Boodlea Montagnei* might be assigned to either *Boodlea* or *Microdictyon*, the habit characteristic of the latter being the more common. As was pointed out by Egerod (1952, p. 332), however, the special anastomosing cells or tenacula, present in this species, are a feature characteristic of *Boodlea* and for this reason she transferred it from *Microdictyon* to *Boodlea*. We concur with this decision.

From the accounts of Reinbold (in Weber-van Bosse, 1913, p. 73) and Setchell (1926, p. 151; 1929, p. 578; 1935, p. 131), it is seen that they were impressed by the correspondence between *Microdictyon Montagnei* and *Boodlea paradoxa*. Examination of original material of these two taxa (in the Herbarium of the University of California) and of the accounts of Reinbold and Setchell has led us to conclude that these two species are the same. *B. paradoxa* is accordingly reduced to the status of synonym under *B. Montagnei*.

Setchell (1929, pp. 573, 574) has discussed the nomenclatural history of *Boodlea Montagnei*. A diagnosis of the species (as *Microdictyon Montagnei*) was first supplied by J. E. Gray (1866, p. 69), but this diagnosis was so superficial that it barely validates the name. Nonetheless it is sufficient to validate the name and hence give it priority over *Boodlea paradoxa* Reinbold (1905).

Dictyosphaeria Versluysi Weber-van Bosse

Dictyosphaeria Versluysi Weber-van Bosse, 1905, p. 114.

This genus is here reported for the first time from South Africa. The record is based on material collected by Dr Robert J. Rodin in May 1948 at Kosi Bay.

The South African plants of *Dictyosphaeria* are representative of a species with solid thalli and with the cells containing spinulose trabeculae. Egerod (1952, p. 354) has discussed the difficulty of distinguishing between *D. Versluysi*, *D. van-Bosseae*, *D. australis* and *D. Setchellii* (to which complex of species should be added *D. bokotensis* Yamada, 1925), all of which possess solid thalli and spines that project from the cell wall into the cell cavity. On the basis of the coarseness of the thalli the South African plants are referred to *D. Versluysi*. It should be pointed out, however, that spines are not produced in all or most of the cells in the South African plants of this species as they are in specimens from other regions, as for example, those from Hawaii (*cf.* Egerod, 1952). In the South African specimens spines were observed only in the cells toward the base of the thallus.

Valoniopsis pachynema (Martens) Boergesen

Valoniopsis pachynema (Martens) Boergesen, 1934, p. 10, Figs. 1, 2.

Bryopsis pachynema Martens, 1866, p. 24, Pl. 4, Fig. 2.

Valonia pachynema (Martens) Weber-van Bosse, 1913, p. 61.

Valonia confervoides Harvey ex J. Agardh, 1887, p. 100.

This genus is here reported for the first time from South Africa. The material upon which the record is based was collected by Papenfuss and Pocock at Perriers Rocks (near St. Lucia Bay) in July 1938 and consists of only a few somewhat fragmentary specimens. In these specimens the branching is less regular than in those reported on by Boergesen (1934), in which respect they agree better with those illustrated by Okamura (1909, Pl. 65, Figs. 7-10). In general, however, the South African material seems to conform to Boergesen's description of *Valoniopsis pachynema*. The only other known species of *Valoniopsis* is *V. Hancockii* Dawson (1944), which appears to be a distinct taxon.

Boergesen (1934), Feldmann (1938), and Yamada & Tanaka (1938) place *Valoniopsis* in the Anadyomenaceae, but on the basis of the method of branch initiation, we believe that it is more appropriately referred to the Valoniaceae. When a branch is about to be initiated, a minute lenticular cell is cut off along the lateral

wall of the parent filament. This cell increases in diameter and produces an exogenous protruberance which eventually gives rise to a branch with a basal septum which has been present since the inception of the branch and which remains concave to the wall of the parent axis. This method of branch formation recalls the early stages in the ontogeny of the thallus in members of the Valoniaceae and is not like that characteristic of members of the Anadyomenaceae.

Derbesia Hollenbergii Taylor

Derbesia Hollenbergii Taylor, 1945, p. 75, Pl. 1, Figs. 7-9.

The plants that we are referring to this species (previously known only from Ecuador) were collected by Papenfuss and Pocock at Muizenberg (False Bay) in January 1939 and by Pocock at Strandfontein (False Bay) in January 1946, at both of which localities they grew in sand in intertidal pools in association with various other algae such as *Ulva*, *Bryopsis*, and *Champia compressa*.

A distinguishing feature of the South African plants is that the sporangia have a turbinate shape. Apparently, only two species with sporangia of this form have been described: *Derbesia turbinata* Howe et Hoyt (1916, p. 106, Pl. 11, Figs. 10-16) and *D. Hollenbergii* Taylor (loc. cit.).

Derbesia turbinata, to judge from the description of Howe and Hoyt, is a much finer species than the one from South Africa, with filaments that range in diameter from 38 to 53 μ as contrasted with a range of 60 to 140 μ in the South African material. Moreover, the plants from North Carolina are described as containing pyrenoids, which structures appear to be absent in the South African specimens.

In overall dimensions the South African plants compare favorably with the description of *Derbesia Hollenbergii* as given by Taylor and with isotype material of this species in the University of California Herbarium.

Derbesia prolifica Taylor

Derbesia prolifica Taylor, 1945, p. 75, Pl. 2, Figs. 1-6.

The plants that we are referring to this species (heretofore known only from Ecuador) were collected at Umhlanga Rocks (north of Durban) by Papenfuss and Pocock in July 1938. They occurred in an intertidal pool and were epiphytic on other algae.

Except that the filaments are longer, measuring to 8 cm in length, the South African material seems to agree with *Derbesia prolifica* as described by Taylor. The plants contain an abundance of globose (rarely pyriform) sporangia borne on short pedicels. The sporangia are produced unilaterally along the fairly stout (115-150 μ diam.) filaments. The South African specimens differ from those from Ecuador in being very sparsely branched.

Other species with more or less similarly-shaped sporangia that came under consideration in the determination of the South African specimens are: *Derbesia novae-zelandiae* Chapman (1949 p. 498, Fig. 5), *D. minima* Weber-van Bosse (1913, p. 95, Fig. 23; see also Segawa, 1941, p. 254, Fig. 2), *D. tenuissima* (De Notaris) Crouan fr. (1867, p. 133; see also Boergesen, 1925, p. 107, Fig. 45; Hamel, 1931, p. 99, Fig. 23, II-III; Feldmann, 1937, p. 95, Fig. 32, A), and *D. claviformis* (J. Agardh) DeToni (1889, p. 425). The South African material differs from *D. novae-zelandiae* in the greater diameter of the filaments, the larger sporangia, and the absence of filaments that are appressed to one another; from *D. minima* and *D. tenuissima* in the greater overall dimensions of the filaments and the sporangia; and from *D. claviformis* in having stalked instead of sessile sporangia.

Derbesia ryukyuensis Yamada et Tanaka

Derbesia ryukyuensis Yamada et Tanaka, 1938, p. 64, Fig. 5.
Derbesia longifructa Taylor, 1945, p. 74, Pl. 1, Figs. 3-6.

The South African plants that we are referring to *Derbesia ryukyuensis* were collected at Strandfontein (False Bay) where they occurred as tufts on rocks in the intertidal belt. Although specimens that are probably representative of the same species were obtained at this locality

on several occasions, only those collected in February 1937 were fertile and hence determinable with reasonable certainty. The filaments are irregularly branched, to 2·5 cm long and from 35 to 60 μ (occasionally to 95 μ) in diameter. The sporangia are oblong, and on an average about 90 μ in diameter and 150 μ long, exclusive of the pedicel.

Only three of the known species of *Derbesia* are characterized by the possesion of oblong sporangia: *D. ryukyuensis* Yamada et Tanaka (loc. cit.), *D. pacifica* Jao (1937, p. 106, Pl. 12, Figs. 11-13) and *D. longifructa* Taylor (1945, p. 74, Pl. 1, Figs. 3-6). *D. pacifica*, which is known only from the Pacific coast of North America, is smaller in overall dimensions than the plant from South Africa (the diameter of the filaments is 26-49 μ and they taper toward the apex where they may be only 8-11 μ in diameter; the sporangia are 57-68 μ in diameter and 84-100 μ long, exclusive of the pedicel).

The South African material fits the descriptions of both *Derbesia ryukyuensis* (which was described as having filaments that are 0·3 to 1 cm long, simple or sparingly dichotomously or laterally branched, and 35-45 μ in diameter, and stalked sporangia that are 60-77 μ in diameter and 115-154 μ long) and *D. longifructa* (for which Taylor did not give the length of the filaments, but their diameter is 38-75 μ, and the sporangia are 58-90 μ in diameter and 121-180 μ long). To judge from the original descriptions of these two species, we are inclined to believe that they are conspecific and we are, therefore, referring the South African material to *D. ryukyuensis*, which was described first. This species was recently reported by Dawson (1956) from the southern Marshall Islands. Taylor's material of *D. longifructa* was obtained in Ecuador.

Caulerpa brachypus Harvey

Caulerpa brachypus Harvey, 1859, p. 333.
Caulerpa parvifolia Harvey, 1860, Pl. 172.
Caulerpa anceps Harvey ex J. Agardh, 1872, p. 9.
Caulerpa Stahlii Weber-van Bosse, 1898, p. 282, Pl. 22, Figs. 3, 4.
Caulerpa simplex Levring, 1938, p. 13, Pl. 3, Fig. 9.
Caulerpa mauritiana Boergesen, 1940, p. 45, Pl. 3.
Caulerpa brachypus var. *mauritiana* (Boergesen) Boergesen, 1948, p. 32.
Caulerpa brachypus var. *mauritiana* f. *exposita* Boergesen, 1951, p. 8, Fig. 3.

This taxon was reported from South Africa for the first time by Levring (1938), who considered it a new species, *Caulerpa simplex*, of the section Phyllantoideae. Levring believed it to differ from other members of this section in generally having simpler fronds, branching or proliferation occurring but rarely, and blades with an entire (not toothed) margin. The South African plant appeared to be related to *C. parvifolia*, but Levring concluded that it was not the same since its blades were not of the same breadth throughout and were relatively shorter than those of *C. parvifolia*.

Examination of isotype and other South African material of *Caulerpa simplex* has shown that the blades not only may dichotomize or become proliferous but occasionally also form minute teeth along the margins. The autonomy of *C. simplex* thus comes into question.

A comparison of the material of *Caulerpa simplex* now at hand with the published descriptions and illustrations (and material in the University of California Herbarium) of some of the other species of the section Phyllantoideae has led to the conclusion that *C. simplex*, *C. parvifolia*, *C. brachypus*, and *C. mauritiana* are the same species, whose name should be *C. brachypus*.

Although specimens of *Caulerpa brachypus* from Japan, the type region, are generally larger than those from South Africa, it is apparent from the published accounts that this is an extremely variable species. Our concept of this taxon has undergone considerable change in the past fifty years. In 1903 Yendo published his conclusion that *C. brachypus*, *C. anceps*, and *C. Stahlii* were perhaps merely growth forms of the same species. He was of the opinion that environmental and especially seasonal conditions might be responsible for the variation of this species. Weber-van Bosse (1913) accepted the conclusions of Yendo that these three taxa were

representative of the same species, *C. brachypus*. Gilbert (1942) has found, furthermore, that forms representative of the three taxa may be obtained at the same time of the year in the same region.

In 1940 Boergesen described from Mauritius a taxon related to *Caulerpa brachypus* under the name *C. mauritiana*. Later, he (Boergesen, 1946, 1948) also received specimens from Mauritius that he identified with *C. brachypus*. This caused him to re-examine his material of *C. mauritiana* and he arrived at the conclusion that this taxon should be reduced to the status of variety under *C. brachypus*. In view of the great variation of *C. brachypus*, it hardly seems justifiable, however, to retain *mauritiana* even as a variety. It apparently is no more distinctive than some of the other variants of *C. brachypus* that at one time were regarded as autonomous species. Neither does it appear desirable to maintain the forma *exposita* of variety *mauritiana* that was erected by Boergesen in 1951.

On the basis of Weber-van Bosse's (1926) observations that one and the same plant may bear some fronds representative of *Caulerpa brachypus* and others representative of *C. subserrata* Okamura (1897), the possibility exists that even this seemingly clear-cut taxon is merely a growth form of *C. brachypus*. Whether or not the same might be true of *C. biserrulata* Sonder (1871) cannot be said on the basis of present meagre knowledge of this taxon. *C. Ollivieri* Dostál (1929) appears to be more closely related to *C. prolifera* than to the *C. brachypus* complex.

In conclusion, then, it seems justifiable to reduce *Caulerpa simplex* (and the very similar *C. parvifolia* from Australia) to the synonymy of *C. brachypus*. The material of *C. simplex* that Levring had in hand did not show marginal denticulations and proliferation of the blade; but with the discovery of these conditions in subsequently collected South African material and in view of the variation in the shape of the blade from spathulate to ligulate, it is no longer possible to clearly separate this taxon from *C. parvifolia* and the highly variable *C. brachypus*. The plant has been collected in South Africa at several localities along the Natal coast and at Port Elizabeth in the Cape Province.

Caulerpa lanuginosa J. Agardh

Caulerpa lanuginosa J. Agardh, 1872, p. 28.
Caulerpa Lycopodium Harvey, 1858, p. 19, Pl. 37*B* (non J. Agardh, 1847, p. 6).

This plant was previously reported from South Africa by Papenfuss (1952, p. 168) under the name *Caulerpa selago*. A re-examination of the material has shown, however, that it is representative of *C. lanuginosa*. In general appearance, *C. selago* resembles *C. lanuginosa*, but they differ in that the stolon is naked in *C. selago* whereas it is covered with branched wooly hairs in *C. lanuginosa*.

Caulerpa lanuginosa has been obtained at two localities on the east coast of South Africa: at Kosi Bay (leg. Rodin, May 1948) and at the rocks just south of the mouth of the St. Lucia Estuary (Papenfuss and Pocock, July 1938). The South African plants are smaller and finer than those from the Caribbean, the type region, but in other respects agree well with this species. They apparently are not representative of the taxon referred to variety *delicatula* by Weber-van Bosse (1898, p. 305), of which, however, we have not seen material.

Isaac (1956) reports material from Portuguese East Africa under the name *Caulerpa selago*. Inasmuch as the stolon of the plant illustrated in his Fig. 6 is apparently provided with hairs, it appears likely that the species is *C. lanuginosa* instead of *C. selago*.

As regards the nomenclature of this species, it might be remarked that it was first described by Harvey (1858) as *Caulerpa Lycopodium* (under which name Weber-van Bosse, 1898, published her observations on it), but since this binomial had already been used by J. Agardh (1847) for a different plant, J. Agardh in 1872 proposed the name *C. lanuginosa* as a substitute for Harvey's *C. Lycopodium*.

Caulerpa lentillifera J. Agardh

Caulerpa lentillifera J. Agardh, 1837, p. 173.

This species is here reported for the first time from South Africa. The few specimens at hand were collected along the Natal coast by Papenfuss and Pocock in July 1938 at Umhlali (Chaka's Rock) and by Pocock in October 1951 at Reunion Rocks (near Isipingo). The species appears to be rare in South Africa.

Whether or not *Caulerpa lentillifera* should be regarded a variety of *C. racemosa* is a question that may well be considered, as was remarked by Eubank (1946), but this problem had best be attacked by someone who has access to an abundance of living or preserved material. For the present it seems advisable to follow long-standing custom by accepting the taxon as an autonomous species.

Caulerpa racemosa (Forskål) J. Agardh var. *peltata* (Lamouroux) Eubank

Caulerpa racemosa (Forskål) J. Agardh var. *peltata* (Lamouroux) Eubank, 1946, p. 421, Fig. 2*r*, *s*.

Caulerpa peltata Lamouroux, 1809, p. 332.

This widely distributed plant was previously reported from South Africa by Weber-van Bosse (1898, p. 375), who obtained material at Durban. The taxon is usually regarded as a species. Eubank (1946) has assembled the evidence favoring its treatment as a variety. The South African plants studied by us (we have not seen the Weber-van Bosse material) were obtained at various localities along the Natal coast from Umhlanga Rocks (near Durban) in the south to Kosi Bay in the north.

Caulerpa racemosa (Forskål) J. Agardh var. *racemosa*

Caulerpa racemosa (Forskål) J. Agardh, 1872, p. 35.

Fucus racemosus Forskål, 1775, p. 191.

Caulerpa clavifera (Turner) C. Agardh, 1822, p. 437.

Caulerpa racemosa var. *clavifera* (Turner) Weber-van Bosse, 1898, p. 361.

Fucus clavifer Turner, 1808, p. 126, Pl. 57.

Caulerpa racemosa var. *uvifera* (C. Agardh) J. Agardh, 1872, p. 35.

Caulerpa clavifera var. *uvifera* C. Agardh, 1822, p. 438.

Fucus uvifer Turner, 1819, p. 81, Pl. 230 (non Forskål, 1775, p. 192 = *Laurencia uvifera* (Forskål) Boergesen, 1932*a*, p. 12).

The plants here referred to *Caulerpa racemosa* (Forskål) J. Agardh var. *racemosa* have large subglobose to pyriform ramuli (2-5 mm long and 2-5 mm in diam.) and were obtained at a number of localities along the Natal coast. Since the time that Weber-van Bosse (1898) published her monograph on *Caulerpa*, this taxon has been known as *C. racemosa* (Forskål) J. Agardh var. *clavifera* (Turner) Weber-van Bosse, under which name Levring (1938, p. 14) published material from South Africa (determination confirmed by the senior author).

In deciding on the name by which this taxon should be known, the following three names come up for consideration: (1) the basionym of *Caulerpa racemosa*, namely, *Fucus racemosus* Forskål, (2) the basionym of variety *clavifera*, namely, *Fucus clavifer* Turner, and (3) the basionym of variety *uvifera*, namely, *C. clavifera* var. *uvifera* C. Agardh.

Examination (by the senior author) of the types of *Fucus racemosus* Forskål (Copenhagen — type locality the Red Sea) and *Fucus clavifer* Turner (Kew — type locality the Red Sea) revealed that the long-standing belief that these two names apply to the same plant is correct. Hence, in accordance with Article 26 of the Paris Code (1956) the name of this taxon should be *Caulerpa racemosa* (Forskål) J. Agardh var. *racemosa* (without citation of an author's name).

Search for the type of *Fucus uvifer* Turner (1808 — not of Forskål, 1775), which must be considered the type of *Caulerpa clavifera* var. *uvifera* C. Agardh or *C. racemosa* var. *uvifera* (C. Agardh) Weber-van Bosse (as this taxon is currently known), at Kew and the British Museum has not been fruitful. To judge, however, from Turner's illustration of the type, it would seem that there is little justification for recognition of *C. racemosa* var. *uvifera* as a taxon distinct from *C. racemosa* var. *racemosa* and it is here formally reduced.

Caulerpa racemosa (Forskål) J. Agardh var. *turbinata* (J. Agardh) Eubank

Caulerpa racemosa (Forskål) J. Agardh var. *turbinata* (J. Agardh) Eubank, 1946, p. 420, Fig. 2*o-q*.
Caulerpa clavifera (Turner) C. Agardh var. *turbinata* J. Agardh, 1837, p. 173.
Caulerpa racemosa var. *Chemnitzia* (Esper) Weber-van Bosse, 1898, p. 370, Pl. 31, Figs. 5-8.
Fucus Chemnitzia Esper, 1800, p. 167, Pl. 88, Figs. 1, 4-6.
Caulerpa Chemnitzia (Esper) Lamouroux, 1809, p. 332.

This variety was previously reported from South Africa as *Caulerpa Chemnitzia* by Areschoug (1851, p. 7) and Barton (1893, p. 82) and as *C. racemosa* var. *clavifera* by Delf and Michell (1921, p. 95). Barton's record is based on the report of Areschoug, who had material from "Natal Bay" (Herb. Mus. Botan. Stockholm!). The material published by Delf and Michell is in the Bolus Herbarium of the University of Cape Town. This variety is widely distributed in tropical seas. Its known range in South Africa is from Kosi Bay to Port St. Johns.

Caulerpa scalpelliformis (Brown ex Turner) C. Agardh var. *scalpelliformis*

Caulerpa scalpelliformis (Brown ex Turner) C. Agardh, 1822, p. 437.
Fucus scalpelliformis Brown ex Turner, 1811, p. 95, Pl. 174.
Caulerpa scalpelliformis var. *typica* Weber-van Bosse, 1898, p. 287, Pl. 22, Fig. 11*a*, Pl. 23, Figs. 1-4.

This plant was previously reported from South Africa (St. Lucia Bay) by Papenfuss (1943, p. 81) under the name *Caulerpa scalpelliformis* var. *denticulata*.[1] Although the pinnae of the plants from St. Lucia Bay contain no denticulations, the specimens were assigned to variety *denticulata* because in habit they approach this variety more nearly than variety *scalpelliformis* (var. *typica* Weber-van Bosse). With the exception of part of one collection from Kosi Bay (leg. Rodin), the pinnae in South African specimens are uniformly without denticulations. It thus may be more correct to assign the bulk of the South African material to variety *scalpelliformis*. For comments on the variability of this species, reference should be made to the accounts of Weber-van Bosse (1898, p. 288), Svedelius (1906,p. 110), Boergesen (1932b, p. 57) and Rayss (1941, p. 110).

Halimeda cuneata Hering

Halimeda cuneata Hering in Krauss, 1846, p. 214; Kützing, 1849, p. 505; Areschoug, 1851, p. 6; DeToni, 1889, p. 526; Barton, 1893, p. 82; 1901, p. 15, Pl. 1, Figs. 7, 8; Delf & Michell, 1921, p. 95.
Halimeda obovata Kützing, 1858, p. 11, Pl. 25, Fig. I; J. G. Agardh, 1887, p. 86; DeToni, 1889, p. 523.

Halimeda cuneata was described by Hering from material collected by Krauss in Natal Bay (now Durban). A specimen of Krauss's collecting has not yet been located; but as the genus *Halimeda* apparently is represented in South Africa by only one species, there is no question about the identity of Hering's species. Barton (1901, p. 15) saw a Krauss specimen but she did not state in which herbarium it is conserved. Search for such a specimen in the herbaria of the British Museum and Kew has been unsuccessful.

Kützing (1858) described a second species, *Halimeda obovata*, from South Africa, whose type likewise has not been located — it is not in his collection in the Rijksherbarium at Leiden nor does it appear to be in the collection of Sonder (who furnished the specimen) in the National Herbarium of Victoria (Melbourne). In agreement with Barton (1901), we regard this taxon as the same as *H. cuneata*. This interpretation is based on Kützing's illustration of the plant, which shows that the segments are stalked, a feature characteristic of *H. cuneata*. Furthermore, Kützing's type was obtained at Algoa Bay, whence we have seen only

1 Kützing (1843, p. 308) reported *Caulerpa scalpelliformis* from South Africa, but in subsequent accounts of it (1849, p. 496; 1857, p. 3) he gave West Africa as the only locality in Africa. No specimens from South Africa occur in his herbarium. It seems likely, therefore, that he erred in originally crediting it to South Africa.

typical *H. cuneata* and which apparently is the southernmost known locality for *Halimeda* in South Africa. To judge from Kützing's (1849, p. 505; 1857, p. 8) accounts of *H. cuneata*, it is evident that he never saw authentic material of this taxon.

As Barton's (1901) account of the anatomical structure of *Halimeda cuneata* is incomplete and since she considered the species as having an extensive distribution in the Indian and Pacific Oceans, which probably is not the case, it may be useful to give a somewhat full account of our observations on this species. (See also Gilbert, 1947, p. 126, footnote, who remarks: "The writer has not seen material from any locality other than South Africa which proved to be *H. cuneata* although several sets have been studied that were published and distributed as that species from regions other than South Africa . . . Instead the plants usually prove to be *Halimeda discoidea* Decaisne.")

The thallus of *Halimeda cuneata* consists of flat, smooth, cuneate segments or internodes that are joined to one another by a bundle of uncorticated medullary filaments constituting a connecting tissue, which was referred to by Barton as a stalk. The stalk varies in length from about three-tenths (or less) of a millimeter to about one millimeter. Typically the stalk is surrounded at its base by a conspicuous ring of tissue, referred to by Barton as a cushion, that develops from the segment below. This ring is structurally similar to a segment in that it contains well-developed subcortical and cortical utricles. From Barton's account the impression is gained that a connecting tissue (stalk) and a collar are by no means a constant feature of *H. cuneata*. These structures constitute a very conspicuous and characteristic feature, however, of most specimens of *H. cuneata* — only rarely are the segments sessile and a large majority of them possess a terminal collar. These nodal characters thus appear to be sufficiently unique and constant to set *H. cuneata* apart as a clear-cut species.

Our observations on the detailed structure of the node or stalk are not in complete agreement with the account of Barton. When a segment initiates a new segment at its apex, the central group of the longitudinal medullary filaments become profusely branched and interwoven and give rise to a raised collar (ring) of tissue at the summit of the segment. As has been mentioned, this collar is composed of subcortical and cortical utricles. Some of the filaments ascend through the collar and remain unbranched and uncorticated until they start producing the new segment. These exposed filaments constitute the node or stalk. In addition to being simple, these nodal filaments differ from the medullary filaments of a segment in that they are much more regularly cylindrical or subtorulose in shape and their walls are considerably thicker, attaining a thickness of 7-9 μ as contrasted with 2-4 μ farther down in the internode. Both Barton (1901) and Howe (1907, p. 498) have observed the fusion of the medullary filaments in twos or threes at the apex of the joint in *Halimeda cuneata*. We have observed a similar fusion of the medullary filaments at the summits of the segments, but in our experience only a few such fusions occur in each segment — they are not a conspicuous feature of this species. Barton's Fig. 11a (Pl. 1) which allegedly depicts such a fusion of medullary filaments is, in our opinion, almost certainly based on misinterpretation of the section. The open pits probably represent the places of issuance of lateral branches from the central filaments instead of places of fusion of filaments.

Finally, as Gilbert (1947) has pointed out, Barton failed to take the morphology of the subcortex into account. Her Fig. 14 (Pl. 1) is scarcely adequate for purposes of identification. Howe (1907, p. 499) was the first to point out that ". . . the characters of the peripheral utricles and the utricles of the subcortical layer . . . in most species, at least, offer peculiarities of as much constancy and value as do the nodal filaments . . . these elements possess characters of taxonomic value of which any final and complete system of classification must take cognizance." In *Halimeda cuneata* the subcortex consists of one to three (or four) layers of globose to subglobose or irregularly inflated bladders whose diameter is 30-60 μ. The more peripheral bladders issue in several planes and irregularly from the preceding ones. The

cortical utricles are stalked and are borne on the ultimate subcortical utricles. They have a diameter of 25-40 μ and range in length from 60 to 90 μ, exclusive of the stalk. These superficial utricles adhere to one another for half to three quarters their length even after decalcification.

Acetabularia Moebii Solms-Laubach

Acetabularia Moebii Solms-Laubach, 1895, p. 30, Pl. 4, Fig. 1.
Acetabularia Wettsteinii Schussnig, 1930, p. 338, Figs. 1-4.

The occurrence of *Acetabularia* in South Africa was first reported by Levring (1938). He had only one specimen, however, and was unable to identify it as to species. The present record of *A. Moebii* is based on two collections. Dr Robert J. Rodin collected three specimens on a small coral reef about one mile south of Kosi Bay in May 1948 and Dr Mary A. Pocock sent three specimens that she obtained on the side of a deep pool at Hibberdene (Natal) in October 1951.

The overall features of the South African plants conform to those of the plants from Hawaii referred to *Acetabularia Moebii* by Egerod (1952, p. 411). Their dimensions exceed those of this species as reported from most regions, but are within the limits of the plants from the Mediterranean that until recently had passed as *A. Wettsteinii* (cf. Feldmann & Feldmann, 1947, p. 81).

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APOMIXIS IN *BOTHRIOCHLOA, DICHANTHIUM* AND *CAPILLIPEDIUM*

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The three genera of grasses discussed in this report are closely related members of the tribe Andropogoneae, which along with *Eremopogon*, *Euclasta*, and *Pseudosorghum* constitute the section Amphilophiastrae of Stapf. The last three genera are all small with only a few described species and have a limited geographical distribution, whereas *Bothriochloa*, *Dichanthium*, and *Capillipedium* each have several described species and are widely distributed through the tropics and subtropics of the Old World.

Plant breeders have long suspected apomixis in King Ranch Bluestem [*Bothriochloa ischaemum* var. *songarica* (Rupr.) Celarier & Harlan]. This suspicion is further supported by a preliminary embryological study by Hubbard (1950) and a detailed cytological study of the species by Celarier (in press). However, it was only after a crossing technique had been developed (Richardson, unpublished) that conclusive evidence of apomixis could be obtained by means other than detailed embryological studies.

In order to demonstrate conclusively reproduction by apomixis the following conditions must be met:

(a) progenies of distinguishable parents should be of the maternal type,

(b) this maternal inheritance must not be due to self-fertilization,

(c) nor can it be due to complete genetic dominance of the characters of one parent over the other.

These three conditions were studied in turn and the results are presented in this paper.

Materials and Methods

The emasculations and pollinations were made by W. L. Richardson by a method the reliability of which has been demonstrated elsewhere (Richardson, unpublished) and will be discussed later.

Seed harvested from these crosses were handled by our normal laboratory procedures (Celarier & Harlan, 1956) and all seedlings were transplanted to our experimental garden.

Morphological data were taken on all plants, both in the seedling stage and as mature plants. Those plants identical to the maternal accessions in all characters observed were so indicated, but any plant not entirely maternal was analyzed in detail.

Maternal Inheritance

In this study crosses were attempted involving several different types and

species of the three genera considered (Table 1) and a summary of these results are presented below.

Two cytological types of *Dichanthium annulatum* were used as females in crosses with other " annulatum " types and with *D. caricosum*. The progeny from these attempted crosses were from 89·5 per cent to 100 per cent maternal types.

The *D. caricosum* complex was represented in this study by two types that have been designated *typica* and *aristatum* (Celarier & Harlan, 1955). Maternal types ranged from 91·2 to 100 per cent in the progenies.

In the genus *Capillipedium* two species were used. Although only a few crosses were attempted, they included both interspecific and intergeneric combinations. All of the progenies were maternal except for one plant that appeared to be a contamination.

Bothriochloa was used extensively in these studies and female parents included *B. venusta*, two types of the *B. intermedia* complex, one of the *B. pertusa* complex, four types of *B. ischaemum* (Celarier & Harlan, in press), and one unidentified type.

B. venusta was crossed with all three genera and produced two types of offspring: maternal types (Figs. 1, 2, 6; 1, 3, 7; 1, 4, 8), and plants with characteristics of both parents (Figs. 1, 2 and 5). The lowest per cent of maternal types was 78·7 per cent in crosses with *Dichanthium annulatum*.

The *B. intermedia* complex included accessions of both *B. caucasica* and *B. intermedia*. *B. caucasica* was crossed with *D. annulatum* and *C. spicigerum* and although the progenies included only 13 plants, they were all maternal. *B. intermedia* was crossed with four species of *Bothriochloa* and with *C. parviflorum*. In the progenies from these crosses all plants were maternal except one.

In the *B. pertusa* complex, the *insculpta* type was used as a female in a cross with the *typica* type. Only three plants were matured from this cross and they were all maternal.

Two varieties of *B. ischaemum* were used; var. *ischaemum* with $2n=40$ and 60 types, and var. *songarica* with $2n=50$ and 60 types. Each of these types was crossed with several species in both *Dichanthium* and *Bothriochloa* and in one instance with *Capillipedium*. Almost all progenies were 100 per cent maternal and the occasional off type produced showed no obvious paternal characteristics.

One accession of *Bothriochloa* could not be associated with any species familiar to the authors, but was somewhat intermediate between *B. ischaemum* and *B. intermedia*. It was crossed with *B. venusta* and *B. ischaemum* and all of the progeny were maternal in appearance.

A more concise summary of these results is presented in Table 2. All types except *B. venusta* produced above 90 per cent maternal types in the progenies and most were above 95 per cent.

This can be explained only in the following ways:

1. Accidental self-fertilization of extremely homozygous parental material.
2. Complete genetic dominance of all the characters that distinguish the maternal type from the paternal.
3. Apomictic reproduction.

An elaboration of these points is presented below.

Self-Fertilization

Since all of the materials reported on were hand emasculated and hand pollinated, the only opportunity for self-fertilization was where the emasculation was faulty. The reliability of the emasculation procedure was checked by using a sexual diploid of *D. annulatum*. This $2n=20$ type crossed with other ploidy types with extreme difficulty. Four thousand four hundred and fourteen florets of this accession were emasculated and crosses were attempted in several combinations. Only four seeds were produced, two of which germinated, and one plant survived to maturity. This one plant was *not* maternal.

On the other hand, when a sister plant or another $2n=20$ accession was used as the pollinator, seed set was very good and a high percentage of mature plants was produced.

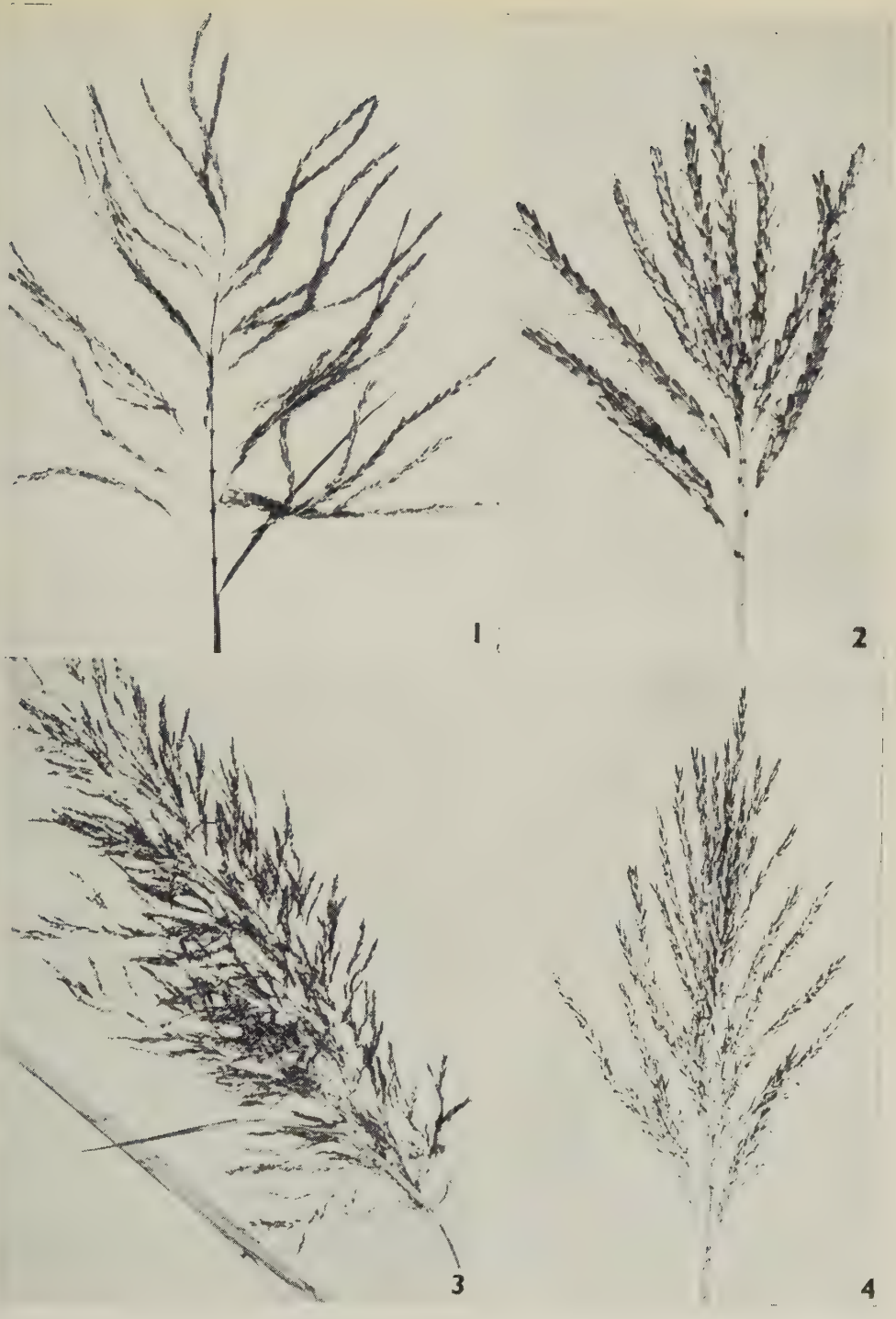
The emasculation technique is, therefore, highly reliable, and the few selves that

TABLE 1 — APOMIXIS IN THE AMPHILOPHIASTRAE

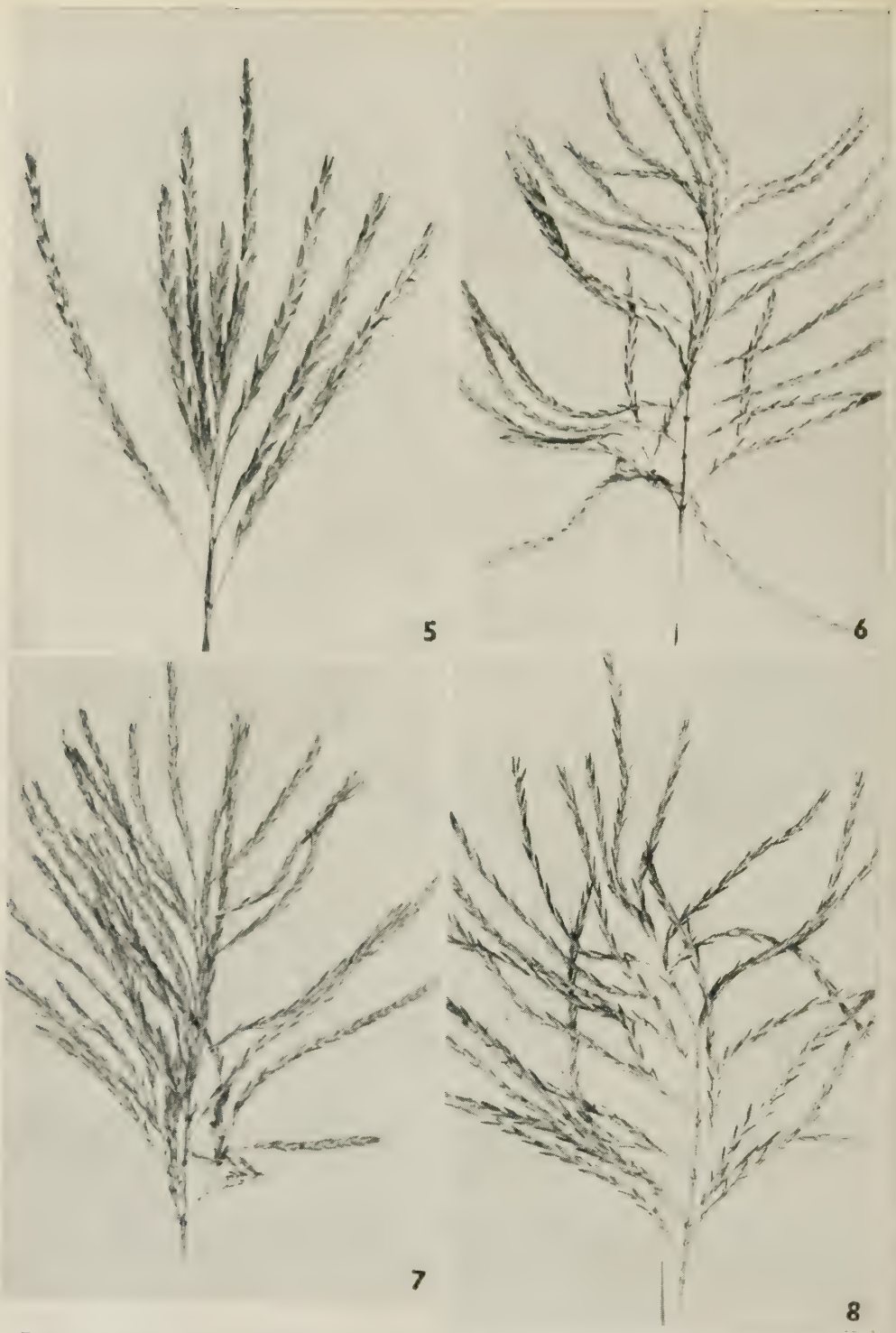
♂	No. PLANTS GROWN	No. PLANTS MATERNAL	PERCENTAGE OF MATERNAL	No. CROSSES INVOLVED	No. STUDIED CYTOLOGICALLY	2*n*
Dichanthium annulatum 2*n* = 40 as ♀ X						
D. annulatum 2*n* = 20	19	17	89·5	2	2	40
D. annulatum 2*n* = 40	181	180	99·4	5	0	
D. annulatum 2*n* = 60	13	12	92·3	3	0	
D. caricosum aristatum 2*n* = 40	2	2	100·0	2	0	
Dichanthium annulatum 2*n* = 60 as ♀ X						
D. annulatum 2*n* = 20	2	2	100·0	1	0	
D. annulatum 2*n* = 40	14	14	100·0	2	0	
D. caricosum 2*n* = 40	2	2	100·0	1	0	
Dichanthium caricosum typica 2*n* = 40 as ♀ X						
D. caricosum typica 2*n* = 40	4	4	100·0	1	0	
D. caricosum aristatum 2*n* = 40	34	31	91·2	2	0	
D. annulatum 2*n* = 60	1	1	100·0	1	0	
Dichanthium caricosum aristatum 2*n* = 40 as ♀ X						
D. caricosum typica 2*n* = 40	90	87	96·7	1	0	
Bothriochloa venusta 2*n* = 40 as ♀ X						
D. annulatum 2*n* = 40	75	59	78·7	1	1	40
B. pertusa insculpta 2*n* = 60	10	10	100·0	3	1	40
B. intermedia 2*n* = 40	2	2	100·0	2	0	
B. intermedia 2*n* = 60	26	25	96·2	1	2	40
B. ischaemum 2*n* = 50	1	0	0·0	1	0	
B. ischaemum 2*n* = 60	6	5	83·3	1	0	
Bothriochloa sp.	2	2	100·0	1	0	
Capillipedium spicigerum 2*n* = 40	1	1	100·0	1	0	
Capillipedium parviflorum 2*n* = 40	1	1	100·0	1	0	
Bothriochloa caucasica 2*n* = 40 as ♀ X						
D. annulatum 2*n* = 40	10	10	100·0	2	0	
Capillipedium spicigerum 2*n* = 40	3	3	100·0	1	0	
Bothriochloa intermedia 2*n* = 40 as ♀ X						
B. venusta 2*n* = 40	18	18	100·0	2	0	
B. intermedia 2*n* = 40	81	81	100·0	5	0	
B. ischaemum 2*n* = 60	2	1	50·0	1	1	40
B. pertusa insculpta 2*n* = 60	1	1	100·0	1	1	40
C. parviflorum 2*n* = 40 irreg.	7	7	100·0	1	1	40 (reg.)
Bothriochloa pertusa insculpta 2*n* = 60 as ♀ X						
B. pertusa 2*n* = 40	3	3	100·0	1	0	
Bothriochloa ischaemum 2*n* = 40 as ♀ X						
D. annulatum 2*n* = 40	8	8	100·0	2	0	
B. venusta 2*n* = 40	2	2	100·0	1	0	
B. intermedia 2*n* = 40	1	1	100·0	1	0	
B. pertusa 2*n* = 40	4	4	100·0	2	0	
B. pertusa insculpta 2*n* = 60	2	2	100·0	1	1	40
B. ischaemum 2*n* = 60	8	8	100·0	1	1	40
C. parviflorum 2*n* = 40 irreg.	1	1	100·0	1	0	

TABLE 1 - APOMIXIS IN THE AMPHILOPHIASTRAE (*Contd.*)

♂	No. PLANTS GROWN	No. PLANTS MATERNAL	PERCENTAGE OF MATERNAL	No. CROSSES INVOLVED	No. STUDIED CYTOLOGICALLY	2*n*
Bothriochloa ischaemum 2*n* = 50 as ♀ X						
D. annulatum 2*n* = 40	3	3	100·0	1	0	
B. venusta 2*n* = 40	40	40	100·0	1	1	50
B. intermedia 2*n* = 40	43	43	100·0	3	1	50
B. pertusa 2*n* = 40	15	15	100·0	1	0	
B. pertusa insculpta 2*n* = 60	1	1	100·0	1	1	50
B. radicans 2*n* = 40	5	4	80·0	1	0	
Bothriochloa ischaemum 2*n* = 60 (European type) as ♀ X						
D. annulatum 2*n* = 40	26	26	100·0	1	1	60
B. venusta 2*n* = 40	35	35	100·0	1	2	60
B. intermedia 2*n* = 40	45	45	100·0	4	0	
B. pertusa insculpta 2*n* = 60	28	28	100·0	1	0	
B. ischaemum Oriental 2*n* = 60	15	15	100·0	1	0	
B. radicans 2*n* = 40	13	13	100·0	1	0	
B. pertusa 2*n* = 40	24	24	100·0	2	0	
Bothriochloa ischaemum 2*n* = 60 (Formosa) as ♀ X						
D. annulatum 2*n* = 40	17	17	100·0	1	0	
B. venusta 2*n* = 40	20	20	100·0	1	1	60
B. intermedia 2*n* = 40	25	25	100·0	3	0	
B. intermedia 2*n* = 60	2	2	100·0	1	0	
B. pertusa 2*n* = 40	3	3	100·0	1	0	
B. ischaemum European 2*n* = 60	11	10	90·9	1	0	
B. ischaemum Oriental 2*n* = 60	55	55	100·0	1	0	
B. radicans 2*n* = 40	18	18	100·0	1	0	
Bothriochloa ischaemum 2*n* = 60 (Triangle City, China) as ♀ X						
D. annulatum 2*n* = 40	33	33	100·0	1	1	60
B. venusta 2*n* = 40	57	57	100·0	1	1	60
B. intermedia 2*n* = 40	53	53	100·0	2	0	
B. pertusa 2*n* = 40	3	3	100·0	1	0	
B. pertusa insculpta 2*n* = 60	2	2	100·0	1	0	
B. ischaemum 2*n* = 40	1	1	100·0	1	1	60
B. ischaemum 2*n* = 50	6	6	100·0	1	1	60
B. ischaemum 2*n* = 60	33	33	100·0	1	0	
B. radicans 2*n* = 40	4	4	100·0	1	0	
Bothriochloa sp. 2*n* = 40 as ♀ X						
B. venusta 2*n* = 40	15	15	100·0	1	0	
B. ischaemum 2*n* = 50	1	1	100·0	1	0	
B. ischaemum 2*n* = 60	1	1	100·0	1	1	40
Capillipedium parviflorum 2*n* = 40 irreg. as ♀ X						
B. pertusa insculpta 2*n* = 60	1	1	100·0	1	1	2*n* = 40 (irreg.)
Capillipedium spicigerum 2*n* = 40 as ♀ X						
B. intermedia 2*n* = 60	1	1	100·0	1	1	40
C. parviflorum 2*n* = 40	19	18	94·7	2	0	



FIGS. 1-4 — Inflorescence of the female parent *Bothriochloa venusta* and three male parents. Fig. 1. *Bothriochloa venusta* with large open panicle. × 0·4. Fig. 2. *Dichanthium annulatum* with short digitate inflorescence. × 1·0. Fig. 3. *Capillipedium parviflorum* with very long compact inflorescence and racemes with few joints. × 0·5. Fig. 4. *Bothriochloa intermedia* with small, compact, cone-shaped inflorescence. × 0·5.



FIGS. 5-8 — Progeny of these crosses in which *B. venusta* was the female parent. Fig. 5. *B. venusta* × *D. annulatum* — a hybrid plant with characteristics of both parents. × 1·0. Fig. 6. *B. venusta* × *D. annulatum* — maternal type. × 0·5. Fig. 7. *B. venusta* × *C. ρ insularum* — maternal type. × 0·5. Fig. 8. *B. venusta* × *B. intermedia* — maternal type. × 0·5

TABLE 2 — PER CENT MATERNAL TYPES RECOVERED FROM TOTAL OF CROSSES WITH VARIOUS MALE SOURCES

	$2n$	No. plants grown	No. plants maternal	% maternal plants
D. annulatum	40	215	211	98·1
D. annulatum	60	18	18	100·0
D. caricosum typica	40	39	36	92·3
D. caricosum aristatum	40	90	87	96·7
Capillipedium parviflorum	40	1	1	100·0
C. spicigerum	40	20	19	95·0*
B. venusta	40	124	105	84·7
B. caucasica	40	13	13	100·0
B. intermedia	40	109	108	99·1*
B. pertusa insculpta	60	3	3	100·0
B. ischaemum ischaemum	40	26	26	100·0
B. ischaemum ischaemum	60	186	186	100·0
B. ischaemum songarica	50	107	106	99·1*
B. ischaemum songarica	60	343	342	99·7*
Bothriochloa sp.	40	17	17	100·0

*The four plants indicated were not maternal but neither did they appear to be hybrids. One was probably a contamination, the other three were aberrents but with no paternal characteristics.

might have been produced can in no way explain the high frequency of maternal types that have been recovered.

In addition to this, several of the accessions studied (i.e. $2n=60$ *D. annulatum*, $2n=60$ *B. pertusa insculpta*, $2n=50$ and $2n=60$ *B. ischaemum*, and the $2n=40$ *Capillipedium parviflorum*) are so irregular in their cytological behaviour that few gametes could be expected to be produced with a completely balanced chromosome number (Celarier, in press; Celarier, Mehra, & Wulf, unpublished). In such materials it is inconceivable that uniform, maternal progenies could be obtained through self-fertilization. It is not even likely that constant chromosome numbers would be maintained in the population let alone a degree of homozygosity that would yield uniform progenies.

Dominance of Maternal Characters

If the maternal types in the progenies of these attempted crosses are due to genetic dominance of maternal characters, it is expected that crosses involving accessions with different chromosome numbers would have intermediate numbers, and that reciprocal crosses would have the same dominance and therefore the same phenotypic expression in their progenies.

Several crosses were made in which the parents had different chromosome numbers (Table 1), and the progenies of many of these were studied cytologically.

One cross with the female $2n=40$ and the male $2n=20$ was studied. The progeny was $2n=40$.

Nine attempted crosses were studied where the female was $2n=40$ and the male $2n=60$. The progenies of all these had $2n=40$.

Two crosses with $2n=50$ females and $2n=40$ males, and one with a $2n=50$ female and $2n=60$ male, were studied. The progenies of all were $2n=50$.

Seven crosses were studied with $2n=60$ females, six were with $2n=40$ males and one with a $2n=50$ male. The progenies were all $2n=60$.

One cross was studied in which both parents had $2n=40$ but the female was much more regular in chromosome behaviour than the male. The progeny was $2n=40$ and regular.

From these 21 crosses, involving parents of different chromosome conditions, it is apparent that the progenies were all maternal in chromosome number and behaviour.

Reciprocal crosses were made in six combinations and the results are summarized in Table 3. In all cases, a high percentage of maternal types was recovered and no paternal types were found.

The maternal types are, therefore, not due to the genetic dominance of all characters that distinguish the parents, and apomictic reproduction remains as the only explanation for the prevalence of maternal types in the progenies from dissimilar parents.

Apomictic Reproduction

Apomictic reproduction is, therefore, well established for all the species studied of *Bothriochloa*, *Dichanthium* and *Capillipedium*.

Although in this report they have been included in nine complexes, the materials studied have been described as 12 species and one variety as follows: *Dichanthium annulatum* Stapf, *D. papillosum* Stapf, *D. caricosum* A. Camus, *D. aristatum* C. E. Hubb., *Bothriochloa venusta* A. Camus, *B. caucasica* C. E. Hubb., *B. intermedia* A. Camus, *B. glabra* A. Camus, *B. insculpta* A. Camus, *B. ischaemum* Keng, *ischaemum*, *B. ischaemum songarica* Celarier & Harlan, *Capillipedium parviflorum* Stapf, and *C. spigicerum* S. T. Blake.

The exact nature of the mechanism is not yet known but is being investigated and will be reported elsewhere.

There is some evidence suggesting that pollen is necessary in order to produce seed. In several crosses a very poor seed set is obtained, even though the female parent seems to be an obligate apomict. For example crosses between *B. ischaemum* and *B. pertusa*, *Capillipedium spicigerum* and *B. intermedia*, *Dichanthium annulatum* and *D. caricosum* and several others almost never set seed of any kind, apomictically or otherwise (Table 4). On the other hand when suitable pollen is applied, a good seed set is obtained. It is most likely that these materials are pseudogamous and that pollen or a certain kind

TABLE 3 — MATERNAL TYPES RECOVERED IN RECIPROCAL CROSSES

Cross	No. plants	Plants maternal		No. plants paternal
		No.	%	
D. caricosum typica × *D. caricosum aristatum*	33	30	90·9	0
D. caricosum aristatum × *D. caricosum typica*	90	87	96·7	0
B. intermedia × *B. venusta*	18	18	100·0	0
B. venusta × *B. intermedia*	33	31	93·9	0
B. ischaemum × *B. venusta*	35	35	100·0	0
B. venusta × *B. ischaemum*	6	5	83·3	0
Bothriochloa sp. A-4028 × *B. venusta*	15	15	100·0	0
B. venusta × Both. sp. A-4028	2	2	100·0	0
B. ischaemum v. ischaemum × *B. ischaemum v. songarica*	28	28	100·0	0
B. ischaemum v. songarica × *B. ischaemum v. ischaemum*	11	10	90·9	0
B. ischaemum 2582 × *B. ischaemum* 1347	55	55	100·0	0
B. ischaemum 1347 × *B. ischaemum* 2582	33	33	100·0	0

TABLE 4 — SEED SET AND GERMINATION IN CERTAIN INCOMPATIBLE CROSSES

Cross	No. florets emasc.	Seed set		Germination	
		No.	%	No.	%
B. ischaemum × *B. pertusa*	1383	46	3·30	43	93·5
Cap. spicigerum × *B. intermedia*	227	1	0·44	1	100·0
D. annulatum × *D. caricosum*	2012	29	1·44	5	17·2

is required to stimulate seed production. It is possible that these phenomena may be of some value in determining relationships even where crosses cannot be made.

Discussion and Conclusions

In this group of materials there is considerable variation in the degree of apomixis. The $2n=20$ types of *D. annulatum* are apparently completely sexual (preliminary studies suggest that the $2n=20$ *D. sericeum* is also sexual) and considerable variation in the frequency of sexuality is seen in the materials reported (Table 2).

From these data (Table 2) it is seen that no hybrid types were recovered in *Capillipedium* and *Bothriochloa* except in *B. venusta*, and that apparent hybrids were recovered in all *Dichanthium* types except the $2n=60$ *D. annulatum*. Although the sampling in some cases is inadequate, several tentative conclusions may be drawn:

1. Both *Bothriochloa* and *Dichanthium* are strongly apomictic, but there is a real difference between the two. *Dichanthium* is considerably more sexual than *Bothriochloa* at the tetraploid level and facultative apomicts are common.

2. Only one species of *Bothriochloa* shows any indication toward sexuality. This species, *B. venusta*, is of special interest because of its morphological appearance. The absence of a groove in the pedicel of the pedicellate spikelet in this species is a *Dichanthium* character whereas other characteristics of the species are *Bothriochloa*. Therefore, this species appears to bridge the morphological gap between the two genera, and is likely to be itself of a hybrid origin.

3. Although the basic number for the tribe may be five (Garber, 1950; Celarier, 1956, 1957) there is little doubt but that $n=10$ is the basic building material for much of the tribe. Those members of *Dichanthium* with $n=10$ all appear to be sexual (i.e. *D. annulatum*, and *D. sericeum*), and it is possible that all such functional diploids ($2n=20$) in the Amphilophiastrae are sexual (*see* Stebbins, 1950, for discussion of agamic complexes).

4. In the Old World Amphilophiastrae the $2n=60$ types are mostly extremely irregular in meiotic behaviour (Celarier, in press; Celarier, Mehra & Wulf, unpublished). In these materials obligatory apomixis would have a significant selective advantage and is, in fact, the method of reproduction encountered.

Thus we see the Amphilophiastrae as a polyploid complex with both sexuality and apomixis and the latter both facultative and obligatory. However, even with obligatory apomixis there is still a possibility of genetic exchange since these materials are pseudogamous and pollen in all cases studied seems to be functional. Pentaploid and hexaploid plants with highly irregular meiotic divisions may still serve as male parents in crosses with suitable facultative sexual females.

Summary

Crosses were attempted in 101 different combinations involving several species of *Bothriochloa*, *Dichanthium*, and *Capillipedium*. Progenies from these crosses were grown and frequencies of maternal types were calculated.

All species studied had above 90 per cent maternal types, except *B. venusta* which had 84·7 per cent, and most were above 95 per cent.

It was shown by analysis of sexual accessions that the emasculation technique was sufficiently reliable that these maternal types could not have been due to accidental selfing.

By checking the cytology of the progeny in certain crosses where the parents had different chromsome conditions, it was shown that they were also maternal, and reciprocal crosses gave maternal inheritance regardless of which parent was the female. From this it was concluded that the maternal inheritance could not be explained as genetic dominance.

It was concluded that this maternal inheritance was due to apomictic reproduction and data were presented to show that these materials are probably pseudogamous.

Twelve species of three genera are, therefore, shown to be apomictic and are added to the fast growing list of apomicts in the Gramineae.

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THE GENUS *BANGIOPSIS* SCHMITZ FROM SOUTH INDIA

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The writer collected a small filiform red alga near Mangalore on the west coast of South India in October 1946. Later on, in 1954, the same alga was collected also at Mahabalipuram, near Madras, on the east coast of South India. The alga of both these collections proved to be a species of *Bangiopsis* Schmitz, a little known genus of the Bangiophycidae. A detailed account of this species is given below and this is followed by a consideration of its relationship to *Goniotrichum humphreyi* Collins and the systematic position of the genus.

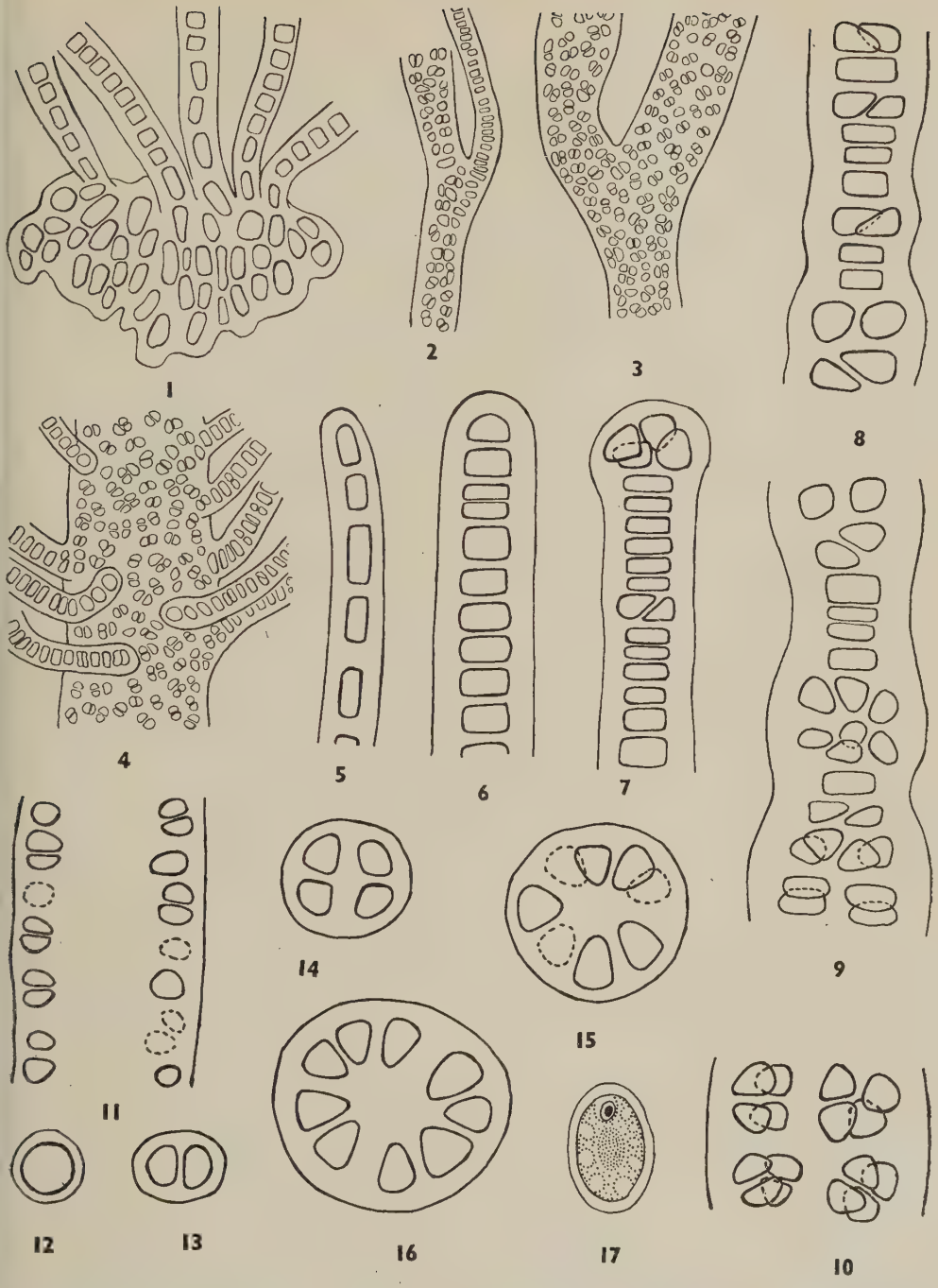
Description

When growing, the alga was attached in small tufts to rocks at high water mark, together with young plants of a species of *Enteromorpha*. Each tuft consists of a number of soft filiform axes measuring up to about ten millimeters in length, springing from a basal disc of cells (Figs. 1, 41). The axes are branched, the branches being mostly in the form of uniseriate proliferations arising from old multiseriate axes (Figs. 2-4).

The young axis is from 8 to 15 μ wide and is composed of a single linear series of discoid to cylindrical cells enclosed by a tubular gelatinous sheath (Figs. 5, 6). Many of the cells show transverse divisions which, presumably, are responsible for growth in length of the axis. Some uniseriate axes in the material were composed of nearly a hundred discoid cells.

In slightly older axes, cells are seen to have divided vertically or obliquely (Figs. 7, 8) at fairly regular intervals. Such divisions may be seen in almost any cell of the axis, including the apical (Fig. 7). In still older axes, these cells divide further, resulting in groups of cells which are separated by uniseriate portions of the axis (Fig. 9). The thallus becomes dilated where these groups of cells occur (Figs. 5-11). In subsequent development, division extends to the intervening uniseriate portions also and ultimately the entire axis, except for a short series of cells at the base, becomes multiseriate (Figs. 3, 37, 40).

The sequence of division seen in transverse sections of the axis is as follows. The young axis shows a single cell in



FIGS. 1-17 — *Bangiopsis subsimplex*. Fig. 1. A tuft of filiform axes arising from a basal disc of cells. × 450. Figs. 2, 3. Two branched axes. × 180. Fig. 4. A multiseriate axis with proliferations. × 180. Fig. 5. A young axis with cylindrical cells. × 900. Fig. 6. A young axis with discoid cells. × 900. Figs. 7-10. Stages in the development of a multiseriate axis as seen in whole mounts. × 900. Fig. 11. Optical section of a multiseriate axis, showing the central non-cellular tract. × 450. Figs. 12-16. Stages in the development of the multiseriate axis, as seen in cross sections. × 900. Fig. 17. A single cell showing details of structure. × 1800.

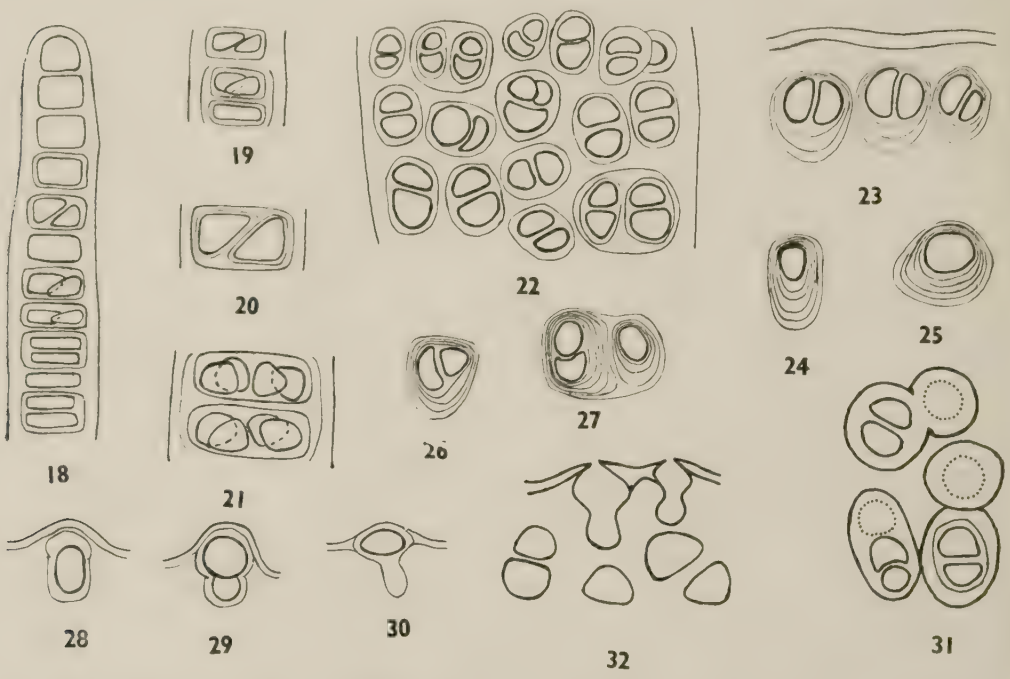
transverse section (Fig. 12). This cell divides vertically or obliquely into two cells (Fig. 13). The two cells then divide radially into four (Fig. 14) and the gelatinous sheath becomes enlarged so as to accommodate the four cells. Subsequently, the cells always divide in any plane perpendicular to the surface (Figs. 15, 16). As a result, the cells increase in number and become arranged in a single peripheral series inside a greatly enlarged sheath (Figs. 11, 15, 16). The maximum width of such fully developed thalli encountered in the material was 120 μ. The central non-cellular tract of these thalli is filled with thin mucilage.

The lowermost parts of the axes remain uniseriate and arise from an irregular disc of somewhat elongate cells (Figs. 1, 41). The alga is attached to the substratum by this basal disc.

The cells in the uniseriate axes are either discoid, 3·2-6·4 μ in length and 6·4-9·6 μ in width, or cylindrical, 6·4-9·6 μ in length and 3·2-6·4 μ in width. In the multiseriate axes, the cells measure 6·4-9·6 μ across and vary in shape around an oval type.

The cell structure is similar to that of many members of the Bangiophycidae. The protoplast is enclosed by a thin firm wall and the cell itself is enclosed by the gelatinous matrix of the thallus. The chromatophore is massive, stellate, enclosing a prominent central pyrenoid. The nucleus is small, peripheral in position, lodged between two arms of the chromatophore (Fig. 17).

During cell division, the daughter protoplasts develop firm walls around themselves, while the wall of the mother cell gelatinizes partially and persists as a mucilaginous sheath enclosing the daughter



FIGS. 18-32 — *Bangiopsis subsimplex*. Fig. 18. A young axis showing modes of division of cells and formation of sheaths. × 900. Figs. 19-21. Stages in the formation of a multiseriate axis, showing formation of successive sheaths. × 900. Fig. 22. Surface view of a part of a multiseriate axis, showing individual sheaths of the cells. × 900. Fig. 23. Part of cross-section of a multiseriate axis showing cells with successive sheaths, enlarging towards the centre of the axis. × 900. Figs. 24-27. Cells from old thallus showing concentric sheaths. × 900. Figs. 28-30. Monospores in various stages of release from their sheaths. × 900. Fig. 31. Part of surface view of thallus showing empty sheaths. × 900. Fig. 32. Margin of thallus showing empty sheaths with opening at the tip. × 900.

FIG. 33. Photograph of type specimen of *Compsopogon subsimplex* Mont. together with label and Montagne's seal. Natural size. Negative No. Com. sub. 1956/1.

cells. The daughter cells formed as a result of one division divide further resulting in two groups of two cells each. Each group has a persistant sheath and the two groups together are enclosed within the first formed sheath derived from the original mother cell (Figs. 19-22). The process is repeated with every subsequent division. This is seen not only in the young uniseriate axis (Figs. 18, 19), but also in the cells of the multiseriate axis (Figs. 22, 44). In a surface view of the latter, cells are associated in pairs or in groups of four (Figs. 2-4, 22, 44), and when suitably stained, are seen to be within a common sheath.

As new sheaths are being formed around the daughter cells of the latest division, the limits of the older sheaths gradually disappear. In the multiseriate axes, this takes place more rapidly towards the centre (Fig. 23). The mucilage derived from these evanescent sheaths, presumably, forms the substance of the central tract of the old thallus.

Very frequently, cells of the old multiseriate thalli which have possibly ceased active division secrete successive new membranes, while the older membranes partially gelatinize forming a succession of sheaths (Figs. 23-27). The outer sheaths become progressively less dense, particularly towards the centre of the axis. These sheaths also contribute to the central non-cellular tract.

No reproductive cells have been recorded so far for *Bangiopsis*, but in the material studied, some old thalli showed a number of cells in various stages of release from their sheaths (Figs. 28-30,

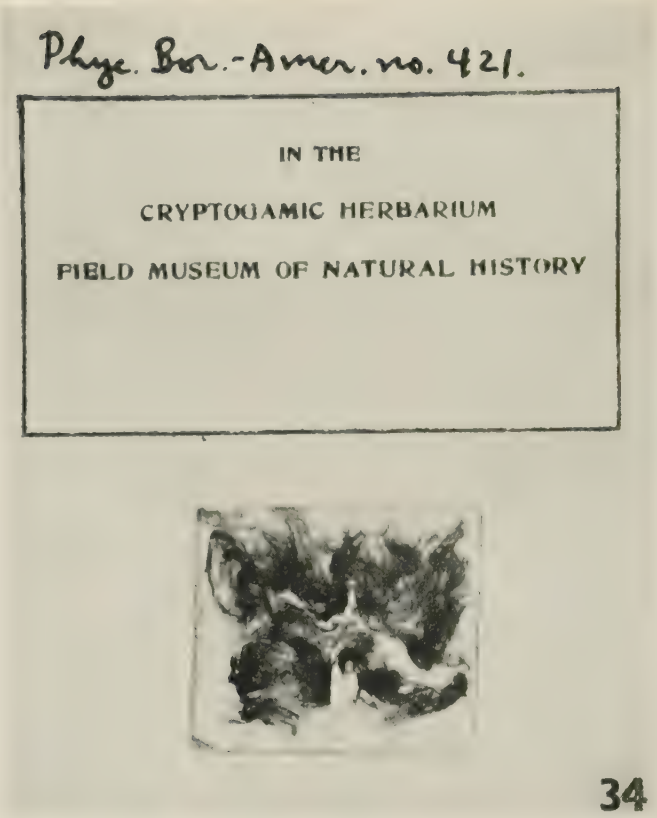


FIG. 34. Photograph of specimen of *Goniotrichum humphreyi* Collins (Phycotheca Boreali-Americana No. 421), together with label. Natural size. Negative No. Gon. hum. 1956/1.

42, 43). There were also seen a number of empty sheaths among the cells of the thallus (Figs. 31, 32, 43). These empty sheaths opened to the outside by a circular perforation through which the contents had evidently squeezed out. The escaping cells appear to have walls. These observations suggest that entire cells of the thallus escaped from their sheaths and from the gelatinous matrix of the thallus by means of a lateral perforation and probably served as monospores. The situation brings to mind the empty sheaths figured by Wille (1900) for *Asterocytis ramosa*.

The Genus *Bangiopsis*

The genus *Bangiopsis* was erected by Schmitz (1896) to accommodate an alga which was previously described by Montagne (1850) under the name of *Compsopogon subsimplex* from material collected by Leprieur from Cayenne in Guiana. This species has not been reported from any other locality since then, although Borgesen (1915-20, p. 10) described an alga from the Danish West Indies under this name. Collins & Hervey (1917) referred Borgesen's alga to *Goniotrichum humphreyi* Collins and subsequently Borgesen (1915-20, p. 445) himself issued a correction agreeing with Collins & Hervey. Hamel (1929) later referred *Goniotrichum humphreyi* to *Bangiopsis*. He also pointed out that the same alga, found in the herbarium of Thuret, had been named by Crouan as *Bangia dumontioides* and a description was published by Schramm & Mazé (1865). Hamel considered the

name given by Crouan as a *nomen nudum* on the ground that the description published by Schramm & Mazé (1865) was inadequate. So, Hamel named the alga *Bangiopsis humphreyi*, adopting the specific epithet used by Collins (Phycotheca Boreali-Americana, No. 421, 1898; 1901). However, Crouan's name was validly published in Schramm & Mazé (1865) and hence is the legitimate name. In the opinion of the writer, Crouan's & Collins' algae should be placed in the genus *Bangiopsis* and the correct name of the alga is *Bangiopsis dumontioides* (Crouan in Schramm & Mazé) comb. nov. [Syn. *Bangia dumontioides* Crouan in Schramm, A, *et* H. Mazé, *Goniotrichum humphreyi* Collins, *Bangiopsis humphreyi* (Collins) Hamel.] Hamel (1929) has also suggested the possibility of *B. humphreyi* being only a form of *B. subsimplex*. In the opinion of the writer, the two are distinct species as will be shown subsequently.

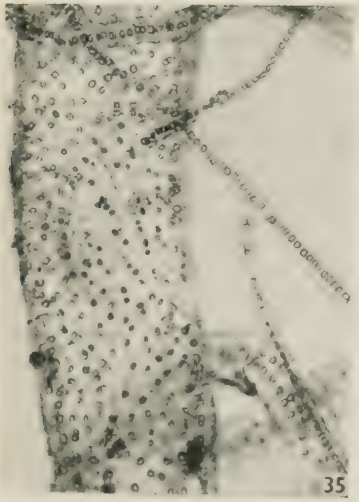
There are thus two species of *Bangiopsis*, *B. subsimplex* (Mont.) Schmitz, so far recorded only from Cayenne, and *B. dumontioides* (Crouan) comb. nov., recorded from various places: as *Goniotrichum humphreyi* Collins, Jamaica (Collins, 1901), Danish West Indies (Borgesen, 1915-20), Bermuda (Collins & Hervey, 1917), Mahabalipuram, South India (Srinivasan, 1946), Brazil (Joly, 1956); as *Bangia dumontioides* Crouan, Guadeloupe (Crouan in Schramm *et* Mazé, 1865).

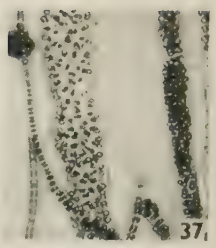
In order to assign the alga described in this paper to its specific epithet, it was thought desirable to examine original specimens of the two known species of *Bangiopsis*. The type material of *Compsopogon subsimplex* Mont., No. 830, of Leprieur's collection was obtained from the Herbarium of the Museum National d'Histoire Naturelle, Paris, through the kind help of Professor R. Lami and M. M. Denizot. The writer was also able to examine the slides of *Compsopogon subsimplex*, prepared by Schmitz from Leprieur's material, at the British Museum of Natural History, London, through the kindness of Mr R. Ross. It was thought desirable to examine *Goniotrichum humphreyi* also since this was mistaken by Borgesen for *Bangiopsis subsimplex*. So, material of *Goniotrichum humphreyi*, No. 421 of Phycotheca Boreali-Americana, was obtained from the Field Museum of Natural History, Chicago, through the kindness of Dr F. Drouet. When the writer examined this material, he was not aware that Hamel had already referred this alga to *Bangiopsis*, but had independently come to the same conclusion.

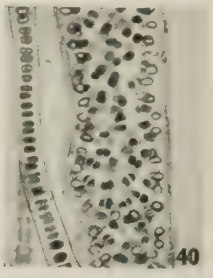
The herbarium sheet bearing the specimen of *Compsopogon subsimplex* has several fragments of the alga, of which the one at the top right-hand corner is said to be the type (Fig. 33). It is described as fruiting. The label on the sheet also refers to the presence of a diatom, *Achnanthes subsessilis* Kütz.

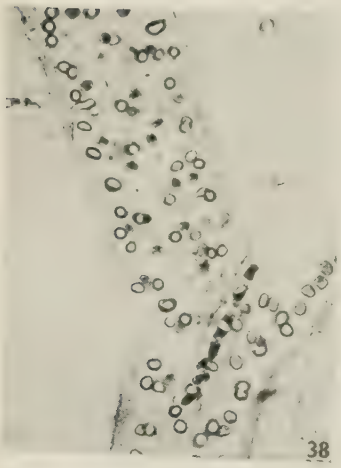
The threads of the alga are comparatively coarse, much branched and adhere but lightly to paper. The exact length to which the alga grew could not be determined as the material was fragmentary, but it is certainly robust. A small fragment of the material was soaked in water, stained in safranin and mounted in karo syrup. On this slide were both uniseriate and multiseriate threads, strikingly similar to those of the form described in this paper (Figs. 36, 37, 39, 40). Measurements of cells as well as axes were made and these agreed closely with those of the South Indian material. The cells in the uniseriate threads were discoid, 3·2-6·4 μ in length and 6·4-9·6 μ in width. Cells of the multiseriate threads measure 6·4-9·6 μ across. Maximum width of old thalli seen in the slide prepared was roughly 150 μ, but wider threads probably exist. Thus the details of microscopic structure are in general agreement with those of the South Indian collection. In fact, the only difference between the two seems to be that the material from Guiana is considerably more robust and profusely branched.

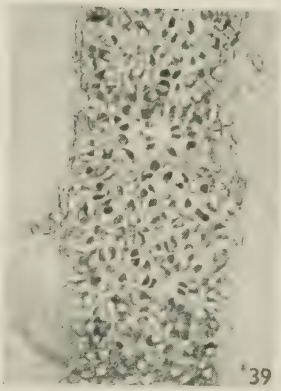
The specimen of *Goniotrichum humphreyi* Collins, No. 421 of Phycotheca Boreali-Americana, consists of a mass of fine threads mounted on a slip of mica (Fig. 34). The threads adhere well to the mica slip. In the fineness of the threads, there is a certain amount of resemblance to the South Indian material, but a marked contrast to the specimen of *Compsopogon subsimplex* from Guiana.

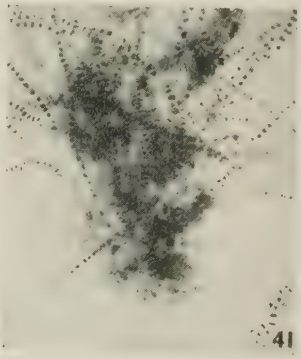


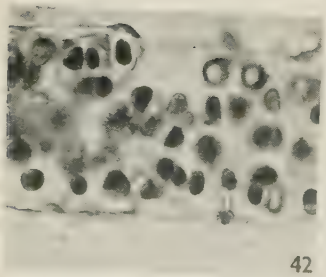


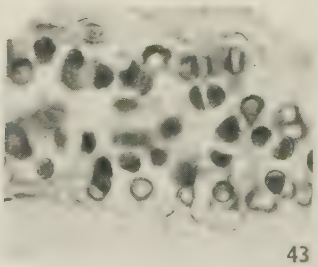


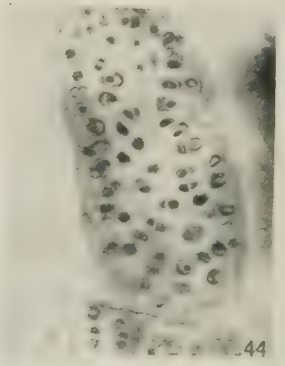












FIGS. 35-44.

A small fragment of the material was removed and soaked in water, stained in safranin and mounted in karo. The slide showed uniseriate and multiseriate threads of the same type seen in the other two collections, so that there is no doubt whatever about *Goniotrichum humphreyi* being a species of *Bangiopsis*. However, this alga differs from the South Indian alga and the material from Guiana in two important characters. The cells of the *Phycotheca* material are, in general, larger and, in the multiseriate threads, they are spaced farther apart (*cf.* Figs. 35, 38 with 36, 37, 39, 40). The cells in the uniseriate threads are either discoid, 4·8-6·4 μ in length and 6·4-11·2 μ in width, or cylindrical, 4·8-11·2 μ in length and 4·8-6·4 μ in width. The cells of the multiseriate threads measure 6·4-12·8 $\mu \times$ 4·8-9·6 μ. The threads are also wider than in the other two collections. In the opinion of the writer, these features are of sufficient importance to distinguish this as a species from *Bangiopsis subsimplex*.

The South Indian material shows great similarity to the type material of *Bangiopsis subsimplex*, though the former has smaller and less freely branched thalli. This may be due to the plants being much younger. Hence the writer identifies the South Indian material with *Bangiopsis subsimplex* (Mont.) Schmitz.

Systematic Position of *Bangiopsis*

Recent classifications of the Bangiophycidae have been based on the morphology of the thallus and the modes of reproduction (Skuja, 1939; Kylin, 1956). As pointed out by Drew (1956 b), our knowledge of the modes of reproduction, especially of the types of spores and the manner of their formation, is very vague and fragmentary. Hence we have to rely on morphological features for assessing the systematic position of the members of the Bangiophycidae.

Beyond gross morphological features, very little is known regarding the structure and development of the thallus in the Bangiophycidae. *Bangiopsis* is a typical example of such imperfection of our knowledge. Schmitz (1896), when delimiting the genus *Bangiopsis*, listed it among the doubtful members of the Bangiaceae. Skuja (1939) places it in the Bangiaceae under the order Bangiales, the only apparent reason for doing so being that it has a more elaborate development of the thallus than members of the Goniotrichales. Kylin (1956) has placed the genus in the Goniotrichaceae under the order Goniotrichales, along with other "filamentous" forms possessing axile, stellate chromatophores with pyrenoids.

This investigation of *Bangiopsis subsimplex* has shown that the thallus should be considered as an organized colony rather than a filament. The cells of *Bangiopsis* are surrounded by individual sheaths lying in a gelatinous matrix which has been derived from still older sheaths. This condition is essentially similar to that in many Schizophyceae such as *Hyella* and *Dalmetella*, where the filamentous thallus is considered to be an organized colony. Each cell of *Bangiopsis* is very like a cell of *Chroothece* (Pascher & Petrova, 1931), in the relation of the cell

←—

FIGS. 35-44 — Fig. 35. *Goniotrichum humphreyi* Collins. Portion of thallus from Phycotheca material stained in safranin and mounted in karo. × 110. Negative No. Gon. hum. 1956/3. Fig. 36. *Compsopogon subsimplex* Mont. Portion of thallus of material in herb. Montagne, stained in safranin and mounted in karo. × 110. Negative No. Com. sub. 1956/2. Fig. 37. *Bangiopsis subsimplex* (Mont.) Schmitz. Portion of South Indian material from Mangalore, stained in safranin and mounted in karo. × 110. Negative No. Bangiop. sub. 1956/1. Fig. 38. *Goniotrichum humphreyi*. Detail of portion of thallus stained in eosine and mounted in karo. × 220. Negative No. Gon. hum. 1956/4. Fig. 39. *Comsopogon subsimplex*. Detail of a portion of filament. × 220. Negative No. Com. sub. 1956/3. Figs. 40-44. *Bangiopsis subsimplex*. Fig. 40. Detail of a portion of Fig. 37. × 220. Fig. 41. A fairly young plant showing a tuft of axes arising from a basal disc of cells. × 125. Negative No. Bangiop. sub. 1956/5. Fig. 42 Monosporangia showing monospores in stages of release. × 490. Negative No. Bangiop. sub. 1956/2. Fig. 43. Two monosporangia with monospores (top) and two empty (bottom). × 490. Negative No. Bangiop. sub. 1956/3. Fig. 44. Portion of mature thallus stained with safranin, showing the sheaths of individual cells as well as groups of cells. × 235. Negative No. Bangiop. sub. 1956/4.

to its sheath and in the manner of its division. The presence of a basal disc, the development of branched axes, the manner of development of the multiseriate from the uniseriate axis and the peripheral arrangement of cells due to the limitation of the planes of cell division are all indicative of an advanced state of organization.

The distinction between a colonial and filamentous organization has been variously interpreted by different writers. Fritsch (1935, p. 17) and Iyengar (1951, p. 27) both consider an alga is filamentous when its cell divides by a transverse wall which unites laterally with the parent wall and the resulting daughter cells cohere permanently. Recently Lund (1956) stated: "The fundamental distinction between a coccoid and a filamentous structure would seem to rest on the fate of the middle lamella of the parent cell. If this is continuous with that of the daughter cells, though they later separate, then the alga is presumably filamentous." But, quite a number of writers have, by implication, used the term "filament" in the sense of a string of cells, whatever its mode of formation. Thus the term has been used to describe various palmelloid algae, colonial diatoms, etc. A precise definition of a filament is, therefore, necessary. In the writer's opinion, Lund (1956) has indicated clearly the essential feature of a filamentous organization. From this point of view, *Bangiopsis* cannot be considered as a filamentous alga, but as a colony showing considerable degree of organization.

Both Skuja (1939) and Kylin (1956) recognize two families in the Goniotrichales, the Goniotrichaceae and the Phragmonemataceae. It has recently been pointed out by Drew (1956 a) that the generic name *Goniotrichum* is an earlier synonym of *Erythrotrichia* Areschoug (a conserved name) and should not have been applied to the algae for which it is currently used, the correct generic name for them being *Stylonema* Reinsch. The ordinal and family names should likewise be Stylonematales and Stylonemataceae respectively. There seems good reason for including *Bangiopsis* in the Stylonemataceae with the genera *Asterocytis* and *Stylonema*[1], with which genera it has much in common. These are the pseudo-filamentous construction of the thallus and axile, stellate chromatophores with pyrenoids. The mode of reproduction recalls that of *Asterocytis*.

Very little is known about the genera included by Kylin in the Phragmonemataceae, so that it is not possible at this juncture to relate *Bangiopsis* to these genera. However, a recent paper by Friedmann (1956) on *Phragmonema* records a number of growth forms of that alga which show some resemblance to the structure seen in *Bangiopsis*.

Feldmann (1955) has merged the Goniotrichales (i.e. the Stylonematales) with the Porphyridiales on the ground that there is no essential difference between them. As this study of *Bangiopsis* has shown, the organization of the thallus is considerably more advanced than in the genera of the Porphyridiales. Moreover, a closer study of reproduction in the unicellular and simple colonial members of the Porphyridiales and the advanced colonial types of the Stylonematales may show differences of such a degree as to support the separation. Indeed the little information so far available supports this view. For these reasons the writer considers that the Stylonematales should not be included in the Porphyridiales.

Summary

Bangiopsis subsimplex (Mont.) Schmitz is recorded here from two stations in South India. The thallus consists of a basal disc of cells from which arise a tuft of filiform axes which are uniseriate to begin with, but become multiseriate later on. The axes are branched, with numerous uniseriate proliferations. The cells of the axis are surrounded by individual sheaths lying in a gelatinous matrix derived from older sheaths, thus showing a colonial organization of an advanced type. In the mature thallus, entire cells escape from their sheaths and probably function as monospores.

1. Kylin also includes the genera *Neevea* and *Goniotrichopsis* in this family, but neither genus has been closely studied.

The South Indian material is compared with type material of both *Compsopogon subsimplex* Mont. and *Goniotrichum humphreyi* Collins. It is concluded that the South Indian alga is the same as *Compsopogon subsimplex* which is the type of *Bangiopsis subsimplex* (Mont.) Schmitz. *Goniotrichum humphreyi* Collins is a species of *Bangiopsis* as already shown by Hamel, but is distinct from *B. subsimplex*. As it is the same alga as the one designated by Crouan as *Bangia dumontioides*, which is an earlier synonym of *G. humphreyi*, a new combination is proposed for this alga, *Bangiopsis dumontioides* (Crouan) comb. nov.

It is pointed out that *Bangiopsis* should be placed in the Stylonemataceae under the order Stylonematales.

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Slides made from type material of *Compsopogon subsimplex* are lodged in the Cryptogamic Laboratory of the Museum National d'Histoire Naturelle, Paris, and those of *Goniotrichum humphreyi* in the Cryptogamic Herbarium of the Field Museum of Natural History, Chicago. The negatives of photographs reproduced in this paper are lodged in the Cryptogamic Botany Laboratory, Manchester University.

Postscript — Since sending the above paper for publication, I had the opportunity of examining a duplicate of the type of Crouan's *Bangia dumontioides* at the Natural History Museum, London, and I am satisfied that it is a *Bangiopsis* and is the same as *Goniotrichum humphreyi* Collins. Going through the specimens of *Baugia* in the museum, I came across one from Ceylon in Ferguson's Ceylon Algae, labelled *Bangia fergusoni* Grun. This proved to be identical with *Bangiopsis subsimplex*. As far as I am aware, the name *Bangia fergusoni* has not been validly published anywhere. Anyway, this name would be a later synonym of *Bangiopsis subsimplex*.

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